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AGRICULTURAL RESEARCH, PUSA.



# ANNALS OF BOTANY

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four Diagrams in the Text

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# CONTENTS.

No. CLXXXV, January, 1933.

	PAGE
THODAY, D., and EVANS, H.—Studies in Growth and Differentiation. IV. The Distribution of Some Solutes in the Tissues of <i>Kleinia articulata</i> . With three Figures in the Text . . . . .	I
IYENGAR, M. O. P.— <i>Ecbalocystopsis indica</i> n.gen. et sp., a New Member of Chlorodendrales. With Figures A to R in the Text . . . . .	21
TINCKER, M. A. H., and DARBISHIRE, F. V.—Studies on the Formation of Tubers and Other Storage Organs. The Influence upon Translocation of the Period of Light and the Supply of Potassium. With Plate I. . . . .	27
HAAS, P., and HILL, T. G.—Observations on the Metabolism of Certain Sea-weeds . . . .	55
DASTUR, R. H., and DESAI, B. L.—The Relation between Water-content, Chlorophyll-content, and the Rate of Photosynthesis in Some Tropical Plants at Different Temperatures. With six Figures in the Text . . . . .	69
GOODWIN, K. M.—A Trisomic <i>Oenothera</i> . With Plate II and one Diagram in the Text . .	89
REES, ETHEL M.—Some Observations on <i>Bifurcaria tuberculata</i> Stackh. With seven Figures in the Text . . . . .	101
BHARADWAJA, YAJNAVALKYA.—Contributions to Our Knowledge of the Myxophyceae of India. With eight Figures in the Text . . . . .	117
BAILEY, I. W.—The Cambium and its Derivative Tissues. VII. Problems in Identifying the Wood of Mesozoic Coniferae. With Plates III and IV . . . . .	145
LEEMANN, A. C.—The Anatomical and Physiological Relation between <i>Hydnora Solmsiana</i> Dinter and its Host. With Plates V and VI . . . . .	159
JORDAN, R. C., and CHIRNALL, A. C.—Observations on the Fat Metabolism of Leaves. II. Fats and Phosphatides of the Runner Bean ( <i>Phaseolus multiflorus</i> ) . . . . .	163
DUERDEN, H.—On the Xylem Elements of Certain Fossil Pteridophyta. With Plate VII .	187
DAS GUPTA, S. N.—Studies in the Genera <i>Cytosporina</i> , <i>Phomopsis</i> , and <i>Diaporthe</i> . III. On the Pathogenicity of <i>Cytosporina ludibunda</i> and its Saltants. With twelve Figures in the Text . . . . .	197

No. CLXXXVI, April, 1933.

SKOVSTED, A.—Cytological Studies in Cotton. I. The Mitosis and the Meiosis in Diploid and Triploid Asiatic Cotton. With Plates VIII to XI and one Figure in the Text . . . . .	227
DICKINSON, CAROLA I.—Some Marine Algal Balls from Tasmania. With three Figures in the Text . . . . .	253
BILLINGS, FREDERICK H.—Development of the Embryo-sac in <i>Phoradendron</i> . With twenty Figures in the Text . . . . .	261
JAMES, W. O., and PENSTON, N. L.—Studies of the Physiological Importance of the Mineral Elements in Plants. IV. The Quantitative Distribution of Potassium in the Potato Plant. With seven Figures in the Text . . . . .	279
DASTUR, R. H., and SAMANT, K. M.—Study of the Products of Photosynthesis in Leaves in Artificial and in Natural Light. With one Figure in the Text . . . . .	295
DEY, P. K.—Studies in the Physiology of the Appressorium of <i>Colletotrichum gloeosporioides</i> . With Plate XII . . . . .	305
BENSON, M.—The Roots and Habit of <i>Heterangium Grievii</i> . With Plate XIII and three Figures in the Text . . . . .	313

	PAGE
EDWARDS, W. N.—On the Cretaceous Fern <i>Paradoxopteris</i> and its Connexion with <i>Weichselia</i> . With Plate XIV and two Figures in the Text . . . . .	317
ISAAC, W. E.—Some Observations and Experiments on the Drought Resistance of <i>Pelvetia canaliculata</i> . With one Figure in the Text . . . . .	343
LEVYNS, M. R.—Sexual Reproduction in <i>Macrocystis pyrifera</i> Ag. With nine Figures in the Text . . . . .	349
METCALFE, C. R.—A Note on the Structure of the Phyllodes of <i>Oxalis Herrerae</i> R. Knuth and <i>O. bupleurifolia</i> St. Hill. With seven Figures in the Text . . . . .	355
SCOTT, D. H.— <i>Archaeopitys Eastmanii</i> . With Plate XV and five Figures in the Text . . . . .	361
GWYNNE-VAUGHAN, H. C. I., and WILLIAMSON, H. S.—The Asci of <i>Lachnea scutellata</i> . With Plates XVI and XVII and eight Figures in the Text . . . . .	375
DAS GUPTA, S. N.—Studies in the Genera <i>Cytosporina</i> , <i>Phomopsis</i> , and <i>Diaporthe</i> . IV. On the Pathogenicity of Certain Strains of <i>Phomopsis</i> and <i>Diaporthe</i> . With five Figures in the Text . . . . .	385
BOWEN, ESTHER J.—The Mechanism of Water Conduction in the Musci considered in Relation to Habitat. With twenty Figures in the Text . . . . .	401

## NOTES.

SINGH, T. C. N.— <i>Cuscuta</i> as a Parasite on a Fern. With four Figures in the Text . . . . .	423
——— —A New Device for Overcoming Electrification in Microtomy. With two Figures in the Text . . . . .	425
GREGORY, F. G.—A Simple Device for Regulating Gas Flow. With one Diagram in the Text . . . . .	427

## No. CLXXXVII, July, 1933.

WARINGTON, KATHERINE.—The Influence of Length of Day on the Response of Plants to Boron. With Plates XVIII and XIX and one Figure in the Text . . . . .	429
BROWNE, ISABEL M. P.—A Fifth Contribution to Our Knowledge of the Anatomy of the Cone and Fertile Stem of <i>Equisetum</i> . With eight Figures in the Text . . . . .	459
JANE, FRANK W.—Teratological Studies in the Tubiflorae. I. Abnormalities in the Flowers of <i>Antirrhinum majus</i> L. With eleven Figures in the Text . . . . .	477
HAWKER, LILIAN E.—The Effect of Temperature on the Geotropism of Seedlings of <i>Lathyrus odoratus</i> . With four Figures in the Text . . . . .	503
SPARROW, F. K., Jr.—The Monoblepharidales. With Plate XX and two Figures in the Text . . . . .	517
HALL, MURIEL P.—An Analysis of the Factors Controlling the Growth Form of Certain Fungi, with Especial Reference to <i>Sclerotinia (Monilia) fructigena</i> . With Plates XXI and XXII and twenty-one Figures in the Text . . . . .	543
EWART, A. J.—On the Preservation of Fungi. With six Figures in the Text . . . . .	579
PHILLIS, E., and MASON, T. G.—Studies on the Transport of Carbohydrates in the Cotton Plant. III. The Polar Distribution of Sugar in the Foliage Leaf. With Plate XXIII and fourteen Figures in the Text . . . . .	585
BOWEN, ESTHER J.—The Mechanism of Water Conduction in the Musci considered in Relation to Habitat. II. Mosses Growing in Damp Situations. With forty-one Figures in the Text . . . . .	635
SEN, P. K., and BLACKMAN, V. H.—On the Conditions Leading to the Injection of Leaves Submerged in Water. With two Figures in the Text . . . . .	663
BOLAS, B. D., and MELVILLE, R.—The Influence of Environment on the Growth and Metabolism of the Tomato Plant. I. Methods, Technique, and Preliminary Results. With fourteen Figures in the Text . . . . .	673

## NOTE.

DAS GUPTA, S. N.—Formation of Pycnidia in <i>Cytosporina ludibunda</i> by the Intermingling of Two Infertile Strains . . . . .	689
--------------------------------------------------------------------------------------------------------------------------------	-----

## No. CLXXXVIII, October, 1933.

	PAGE
GOOD, RONALD.—A Geographical Survey of the Flora of Temperate South America. With two Figures in the Text . . . . .	691
FISHER, R. A.—Selection in the Production of the Ever-sporting Stocks. With one Diagram in the Text . . . . .	727
DICKSON, HUGH.—Saltation Induced by X-rays in Seven Species of Chaetomium. With Plates XXIV and XXV and twenty-seven Figures in the Text . . . . .	735
DU PLESSIS, S. J.—The Life-history and Morphology of <i>Olpidiopsis Ricciae</i> , nov. sp., Infecting <i>Riccia</i> Species in South Africa. With twelve Figures in the Text . . . . .	755
WARE, W. M.—A Disease of Cultivated Mushrooms Caused by <i>Verticillium Malthousei</i> sp. nov. With Plates XXVI and XXVII and six Figures in the Text . . . . .	763
NAYAL, A. A.—A Desert Protosiphon, <i>Protosiphon botryoides</i> (Kütz.) Klebs, var. <i>deserti</i> . With seventeen Figures in the Text . . . . .	787
SAWYER, W. H.—The Development of <i>Entomophthora sphaerosperma</i> upon <i>Rhopobota vacciniiana</i> . With Plates XXVIII and XXIX and one Figure in the Text . . . . .	799
DARLINGTON, C. D.—Chromosome Study and the Genetic Analysis of Species . . . . .	811
STONE, L. H. A.—The Effect of X-radiation on the Meiotic and Mitotic Divisions of Certain Plants. With four Figures in the Text . . . . .	815
SHARPLES, A., and GUNNERY, H.—Callus Formation in <i>Hibiscus Rosa-sinensis</i> L. and <i>Hevea brasiliensis</i> Müll. Arg. With Plates XXX to XXXIV . . . . .	827
CHIPPINDALE, H. G.—The Effect of Soaking in Water on the 'Seeds' of <i>Dactylis glomerata</i> L. With one Diagram in the Text . . . . .	841
COOK, W. R. IVIMEY, and NICHOLSON, W. H.—A Contribution to Our Knowledge of <i>Woronina polycystis</i> Cornu. With sixteen Figures in the Text . . . . .	851
DOWLING, RUBY E.—The Reproduction of <i>Plantago Coronopus</i> : An Example of Morphological and Biological Seed Dimorphism. With three Figures in the Text . . . . .	861
HILL, ARTHUR W.—The Method of Germination of Seeds Enclosed in a Stony Endocarp. With twelve Figures in the Text . . . . .	873
BOWEN, ESTHER J.—The Mechanism of Water Conduction in the Musci considered in Relation to Habitat. III. Mosses Growing in Dry Environments. With twenty-three Figures in the Text . . . . .	889
NOTES.	
EWART, A. J.—On the Presence of Citrinin in <i>Crotalaria crispata</i> . F. v. M. . . . .	913
BANCROFT, H.—On Certain Fossil Plants from East Africa. With two Figures in the Text . . . . .	915
DICKINSON, SYDNEY.—The Nature of Saltation in Certain Species of <i>Helminthosporium</i> and <i>Fusarium</i> . . . . .	920



# INDEX.

## A. ORIGINAL PAPERS AND NOTES.

	PAGE
BAILEY, I. W.—The Cambium and its Derivative Tissues. VII. Problems in Identifying the Wood of Mesozoic Coniferae. With Plates III and IV . . . . .	145
BANCROFT, H.—On Certain Fossil Plants from East Africa. With two Figures in the Text . . . . .	915
BENSON, M.—The Roots and Habit of <i>Heterangium Grievii</i> . With Plate XIII and three Figures in the Text . . . . .	313
BHARADWAJ, Y.—Contributions to our Knowledge of the Myxophyceae of India. With eight Figures in the Text . . . . .	117
BILLINGS, F. H.—Development of the Embryo-sac in <i>Phoradendron</i> . With twenty Figures in the Text . . . . .	261
BLACKMAN, V. H., see SEN, P. K.	
BOLAS, B. D., and MELVILLE, R.—The Influence of Environment on the Growth and Metabolism of the Tomato Plant. I. Methods, Technique, and Preliminary Results. With fourteen Figures in the Text . . . . .	673
BOWEN, E. J.—The Mechanism of Water Conduction in the Musci considered in Relation to Habitat. With twenty Figures in the Text . . . . .	401
The Mechanism of Water Conduction in the Musci considered in Relation to Habitat. II. Mosses Growing in Damp Situations. With forty-one Figures in the Text . . . . .	635
The Mechanism of Water Conduction in the Musci considered in Relation to Habitat. III. Mosses Growing in Dry Environments. With twenty-three Figures in the Text . . . . .	889
BROWNE, I. M. P.—A Fifth Contribution to Our Knowledge of the Anatomy of the Cone and Fertile Stem of <i>Equisetum</i> . With eight Figures in the Text . . . . .	459
CHIBNALI, A. C., see JORDAN, R. C.	
CHIPPINDALE, H. G.—The Effect of Soaking in Water on the 'Seeds' of <i>Dactylis glomerata</i> L. With one Diagram in the Text . . . . .	841
COOK, W. R. IVIMEY, and NICHOLSON, W. H.—A Contribution to Our Knowledge of <i>Woronina polycystis</i> Cornu. With sixteen Figures in the Text . . . . .	851
DARBISHIRE, F. V., see TINCKER, M. A. H.	
DARLINGTON, C. D.—Chromosome Study and the Genetic Analysis of Species . . . . .	811
DAS GUPTA, S. N.—Studies in the Genera <i>Cytosporina</i> , <i>Phomopsis</i> , and <i>Diaporthe</i> . III. On the Pathogenicity of <i>Cytosporina ludibunda</i> and its Saltants. With twelve Figures in the Text . . . . .	197
Studies in the Genera <i>Cytosporina</i> , <i>Phomopsis</i> , and <i>Diaporthe</i> . IV. On the Pathogenicity of certain Strains of <i>Phomopsis</i> and <i>Diaporthe</i> . With five Figures in the Text . . . . .	385
Formation of Pycnidia in <i>Cytosporina ludibunda</i> by the Inter-mingling of Two Infertile Strains . . . . .	689
DASTUR, R. H., and DESAI, B. L.—The Relation between Water-content, Chlorophyll-content, and the Rate of Photosynthesis in Some Tropical Plants at Different Temperatures. With six Figures in the Text . . . . .	69
and SAMANT, K. M.—Study of the Products of Photosynthesis in Leaves in Artificial and in Natural Light. With one Figure in the Text . . . . .	295
DESAI, B. L., see DASTUR, R. H.	
DEY, P. K.—Studies in the Physiology of the Appressorium of <i>Colletotrichum gloeosporioides</i> . With Plate XII. . . . .	305
DICKINSON, C. I.—Some Marine Algal Balls from Tasmania. With three Figures in the Text . . . . .	253

	PAGE
DICKINSON, S.—The Nature of Saltation in Certain Species of <i>Helminthosporium</i> and <i>Fusarium</i> . . . . .	920
DICKSON, H.—Saltation Induced by X-rays in Seven Species of <i>Chaetomium</i> . With Plates XXIV and XXV and twenty-seven Figures in the Text . . . . .	735
DOWLING, R. E.—The Reproduction of <i>Plantago Coronopus</i> : An Example of Morphological and Biological Seed Dimorphism. With three Figures in the Text . . . . .	861
DU PLESSIS, S. J.—The Life-history and Morphology of <i>Olpidiopsis Ricciae</i> , nov. sp., Infecting <i>Riccia</i> Species in South Africa. With twelve Figures in the Text . . . . .	755
DUERDEN, H.—On the Xylem Elements of Certain Fossil Pteridophyta. With Plate VII. . . . .	187
EDWARDS, W. N.—On the Cretaceous Fern <i>Paradoxopteris</i> and its Connexion with <i>Weichselia</i> . With Plate XIV and two Figures in the Text . . . . .	317
EVANS, H., see THODAY, D.	
EWART, A. J.—On the Preservation of Fungi. With six Figures in the Text . . . . .	579
— On the Presence of Citrinin in <i>Crotalaria crispata</i> . F. v. M. . . . .	913
FISHER, R. A.—Selection in the Production of the Ever-sporting Stocks. With one Diagram in the Text . . . . .	727
GOOD, R.—A Geographical Survey of the Flora of Temperate South America. With two Figures in the Text . . . . .	691
GOODWIN, K. M.—A Trisomic <i>Oenothera</i> . With Plate II and one Diagram in the Text . . . . .	89
GREGORY, F. G.—A Simple Device for Regulating Gas Flow. With one Diagram in the Text . . . . .	427
GUNNERY, H., see SHARPLES, A.	
GWYNNE-VAUGHAN, H. C. I., and WILLIAMSON, H. S.—The Asci of <i>Lachnea scutellata</i> . With Plates XVI and XVII and eight Figures in the Text . . . . .	375
HAAS, P., and HILL, T. G.—Observations on the Metabolism of Certain Sea-weeds . . . . .	55
HALL, M. P.—An Analysis of the Factors Controlling the Growth Form of Certain Fungi, with Especial Reference to <i>Sclerotinia (Monilia) fructigena</i> . With Plates XXI and XXII and twenty-one Figures in the Text . . . . .	543
HAWKER, L. E.—The Effect of Temperature on the Geotropism of Seedlings of <i>Lathyrus odoratus</i> . With four Figures in the Text . . . . .	503
HILL, A. W.—The Method of Germination of Seeds Enclosed in a Stony Endocarp. With twelve Figures in the Text . . . . .	873
HILL, T. G., see HAAS, P.	
ISAAC, W. E.—Some Observations and Experiments on the Drought Resistance of <i>Pelvetia canaliculata</i> . With one Figure in the Text . . . . .	343
IYENGAR, M. O. P.— <i>Ecbalocystopsis indica</i> n. gen. et sp., a New Member of <i>Chlorodendrales</i> . With Figures A to R in the Text . . . . .	21
JAMES, W. O., and PENSTON, N. L.—Studies of the Physiological Importance of the Mineral Elements in Plants. IV. The Quantitative Distribution of Potassium in the Potato Plant. With seven Figures in the Text . . . . .	279
JANE, F. W.—Teratological Studies in the <i>Tubiflorae</i> . I. Abnormalities in the Flowers of <i>Antirrhinum majus</i> L. With eleven Figures in the Text . . . . .	477
JORDAN, R. C., and CHIBNALL, A. C.—Observations on the Fat Metabolism of Leaves. II. Fats and Phosphatides of the Runner Bean ( <i>Phaseolus multiflorus</i> ) . . . . .	163
LEEMANN, A. C.—The Anatomical and Physiological Relation between <i>Hydnora Solmsiana</i> Dinter and its Host. With Plates V and VI . . . . .	159
LEVYNS, M. K.—Sexual Reproduction in <i>Macrocystis pyrifera</i> Ag. With nine Figures in the Text . . . . .	349
MASON, T. G., see PHILLIS, E.	
MELVILLE, R., see BOLAS, B. D.	
METCALFE, C. R.—A Note on the Structure of the Phyllodes of <i>Oxalis Herrerae</i> R. Knuth and <i>O. bupleurifolia</i> St. Hill. With seven Figures in the Text . . . . .	355
NAYAL, A. A.—A Desert Protosiphon, <i>Protosiphon botryoides</i> (Kütz) Klebs, var. <i>deserti</i> . With seventeen Figures in the Text . . . . .	787
NICHOLSON, W. H., see COOK, W. R. IVIMEY.	
PENSTON, N. L., see JAMES, W. O.	



	PAGE
PHILLIS, E., and MASON, T. G.—Studies on the Transport of Carbohydrates in the Cotton Plant. III. The Polar Distribution of Sugar in the Foliage Leaf. With Plate XXIII and fourteen Figures in the Text . . . . .	585
REES, E. M.—Some Observations on <i>Bifurcaria tuberculata</i> Stackh. With seven Figures in the Text . . . . .	101
SAMANT, K. M., see DASTUR, R. H.	
SAWYER, W. H.—The Development of <i>Entomophthora sphaerosperma</i> upon <i>Rhopobota vaccini</i> ana. With Plates XXVIII and XXIX and one Figure in the Text . . . . .	799
SCOTT, D. H.— <i>Archaeopitys Eastmanii</i> . With Plate XV and five Figures in the Text . . . . .	361
SEN, P. K., and BLACKMAN, V. H.—On the Conditions Leading to the Injection of Leaves Submerged in Water. With two Figures in the Text . . . . .	663
SHARPLES, A., and GUNNERY, H.—Callus Formation in <i>Hibiscus Rosa-sinensis</i> L. and <i>Hevea brasiliensis</i> Müll. Arg. With Plates XXX to XXXIV . . . . .	827
SINGH, T. C. N.— <i>Cuscuta</i> as a Parasite on a Fern. With four Figures in the Text . . . . .	423
— A New Device for Overcoming Electrification in Microtomy. With two Figures in the Text . . . . .	425
SKOVSTED, A.—Cytological Studies in Cotton. I. The Mitosis and the Meiosis in Diploid and Triploid Asiatic Cotton. With Plates VIII to XI and one Figure in the Text . . . . .	227
SPARROW, F. K., Jr.—The Monoblepharidales. With Plate XX and two Figures in the Text . . . . .	517
STONE, L. H. A.—The Effect of X-radiation of the Meiotic and Mitotic Divisions of Certain Plants. With four Figures in the Text . . . . .	815
THODAY, D., and EVANS, H.—Studies in Growth and Differentiation. IV. The Distribution of Some Solutes in the Tissues of <i>Kleinhovia articulata</i> . With three Figures in the Text . . . . .	I
TINCKER, M. A. II., and DARBISHIRE, F. V.—Studies on the Formation of Tubers and Other Storage Organs. The Influence upon Translocation of the Period of Light and the Supply of Potassium. With Plate I . . . . .	27
WARE, W. M.—A Disease of Cultivated Mushrooms Caused by <i>Verticillium Malthousei</i> sp. nov. With Plates XXVI and XXVII and six Figures in the Text . . . . .	763
WARINGTON, K.—The Influence of Length of Day on the Response of Plants to Boron. With Plates XVIII and XIX and one Figure in the Text . . . . .	429
WILLIAMSON, H. S., see GWYNNE-VAUGHAN, H. C. I.	

## B. ILLUSTRATIONS.

a. PLATES.	I. Formation of Tubers (TINCKER and DARBISHIRE).
	II. <i>Oenothera</i> (GOODWIN).
	III, IV. Mesozoic Coniferae (BAILEY).
	V, VI. <i>Hydnora</i> (LEEMANN).
	VII. Fossil Pteridophyta (DUERDEN).
	VIII-XI. Cytology of Cotton (SKOVSTED).
	XII. Appressorium of <i>Colletotrichum</i> (DEX).
	XIII. Heterangium (BENSON).
	XIV. <i>Paradoxopteris</i> (EDWARDS).
	XV. <i>Archaeopitys</i> (SCOTT).
	XVI, XVII. <i>Lachnea scutellata</i> (GWYNNE-VAUGHAN and WILLIAMSON).
	XVIII, XIX. Response of Plants to Boron (WARINGTON).
	XX. Monoblepharidales (SPARROW).
	XXI, XXII. Growth Form of Fungi (HALL).
	XXIII. Distribution of Sugar in Cotton Leaf (PHILLIS and MASON).
	XXIV, XXV. The Effect of X-rays on Species of <i>Chaetomium</i> (DICKSON).
	XXVI, XXVII. A Disease of Cultivated Mushrooms (WARE).
	XXVIII, XXIX. <i>Entomophthora</i> (SAWYER).
	XXX-XXXIV. Callus Formation (SHARPLES and GUNNERY).

## b. FIGURES.

PAGE

1. Distribution of potassium in transverse sections of different parts of the same *Kleinia* plant (THODAY and EVANS) . . . . . 7
2. Rough diagrammatic representation of relative concentration in different tissues respectively of Ca, K,  $\text{NO}_3$ ,  $\text{PO}_4$ , as indicated by microchemical tests (THODAY and EVANS) . . . . . 17
3. Graphic summary of quantitative data for separated zones (THODAY and EVANS) . . . . . 18
- A-R. *Ecbalocystopsis indica* sp. nov. A, portion of filament showing the rupture of the wall of an earlier generation towards the top; B-E, accumulation of end-pieces of walls of successive generations; F, cell with nucleus; G, cell with chloroplasts and pyrenoids; H, M, division of protoplast; G, I, K, R, cells with chloroplasts; J, intact wall enclosing four cells belonging to two successive generations; L, filament showing loop-formation; N, division into four; O, beginning of loop-formation as a result of cell-division into four; P, cells showing nodular thickening of wall; Q, base of filament with adhesive gelatinous pad (IVENGAR) . . . . . 23
1. Diagram of the apparatus in longitudinal section (DASTUR and DESAI) . . . . . 73
2. Curves illustrating the relationship of water-content and of chlorophyll-content with photosynthesis in the leaves of *Abutilon asiaticum* G. Don., at different temperatures (DASTUR and DESAI) . . . . . 75
3. Curves illustrating the relations of the water-content and of the chlorophyll-content with photosynthesis in the leaves of *Ricinis communis* L. at different temperatures (DASTUR and DESAI) . . . . . 78
4. Curves illustrating the relations of the water-content and of the chlorophyll-content with photosynthesis in the leaves of *Helianthus annuus* L. at different temperatures (DASTUR and DESAI) . . . . . 79
5. Curves illustrating the differences in the seasonal photosynthetic activities of the leaves of *Abutilon asiaticum* G. Don. at different temperatures (DASTUR and DESAI) . . . . . 81
6. Curves illustrating the differences in the seasonal photosynthetic activities of the leaves of *Ricinus communis* L. at different temperatures (DASTUR and DESAI) . . . . . 83
- Diagram of ring and rod chromosomes with attachment of extra chromosome such as would be formed were all possible metaphase chiasmata established and maintained (GOODWIN) . . . . . 93
1. Photograph of a *Bifurcaria* pool at Trestignel, Brittany, showing the exposed tips of the erect fronds (REES) . . . . . 103
2. *B. tuberculata* Stackh. Habit drawing of a plant, showing fertile fronds, young spring fronds and rhizome (REES) . . . . . 104
3. *B. tuberculata*. (a) V.S. through apical cell of young fertile frond. (b) X.S. apical cell. (c) V.S. through young vesicle at tip of erect shoot, showing lysigenous origin. (d) Hyphae teased out from an old attaching disc, showing segmentation at the tips (REES) . . . . . 105
4. *B. tuberculata*. (a) X.S. Young fertile frond near the tip, showing the 'assimilating' and 'storage' systems. (b) Part of V.S. through the conducting system, showing oblique walls and lateral contraction of the cytoplasm (REES) . . . . . 107
5. Part of the rhizome of *B. tuberculata*, showing several attaching discs, one of which is still in contact with a piece of stone (REES) . . . . . 109
6. *B. tuberculata*. V.S. through young conceptacles (with mucilage). (a) Showing complex of cells derived from single initial cell. (b) Beginning of basal hair in a conceptacle slightly older than (a). (c) Still older conceptacle with prominent basal hair (REES) . . . . . 111
7. *B. tuberculata*. X.S. of old receptacle, showing cavity left by 'plugs', also degenerate paraphyses in the neighbourhood of the ostiole (REES) . . . . . 114
1. *CylindrospERMUM muscicola* Kütz. var. *kashmirensis*, var. nov. A, mature filament with heterocysts and spores; B and C, portions of filaments stained with iodine, showing the investments of the cell and the development of the spore; D and E, mature terminal heterocysts; F and G, heterocysts with mature spores (BHARADWAJA) . . . . . 119

## FIGURES.

## PAGE

2. *CylindrospERMUM muscicola* Kütz. var. *kashmirensis* var. nov. A and B, young terminal heterocysts; C, development of spore; D and E, intercalary heterocysts; F, formation of a pair of intercalary heterocysts by the division of a heterocyst-rudiment; G, pair of intercalary heterocysts about to separate; H, filament with a dead cell (BHARADWAJA) . . . . . 121
3. *Aulosira Fritschii* sp. nov. A, portion of a filament; B and C, filaments with intercalary heterocysts; D, filament with mature spores; E and F, germination of mature spores; G, three new trichomes formed by the germination of mature spores, embedded in a linear series within a tubular mass of mucilage (BHARADWAJA) . . . . . 127
4. *Aulosira Fritschii* sp. nov. A and B, filaments with living spores and various stages in their degeneration, also germinating spores; C, filament with immature spores, the end walls of adjacent spores still recognizable; D, germination of immature spores, like those in C; E, new trichome formed by the germination of an immature spore and surrounded by the persisting longitudinal walls of disintegrated spores and the sheaths of the parent-filament; F and G, new trichomes formed by the germination of immature spores within the sheaths of the parent filaments (BHARADWAJA) . . . . . 129
5. *Aulosira prolifica* sp. nov. A, filament with homogeneous sheath; B, filament with sheath differentiated into two regions; C and D, filaments with inner sheath only; E and F, filaments stained with methylene blue, showing the central body; G, terminal portion of an old, and H, of a young trichome; I, trichome with a pad of intercellular substance; J, small fragment of a filament; K, mucilage formed from the sheaths intermingled with escaped hormogones; L, empty sheath with biconcave cell left behind on the escape of hormogones; M, empty sheath with old heterocyst, showing an irregular rupture for the emergence of a hormogone; N, escape of a hormogone by longitudinal splitting of the sheath (BHARADWAJA) . . . . . 133
6. *Aulosira prolifica* sp. nov. A, trichome interrupted by a biconcave cell with a terminal heterocyst at the end of one of the fragments; B-E, filaments showing the formation of heterocysts at the ends of the fragments produced by the dying of cells; F, G, and H, development of heterocysts; I and J, empty sheaths with persisting heterocysts; K and L, filaments breaking adjacent to biconcave cells where the sheath becomes dilated and more or less diffuent (BHARADWAJA) . . . . . 135
7. *Aulosira pseudoramosa* sp. nov. A and B, portions of filaments; C, filament showing rupture of the brittle sheath; D and E, breaking up of trichomes into hormogones; F-L, germination and emergence of hormogones after secretion of a new sheath (BHARADWAJA) . . . . . 139
8. *Aulosira pseudoramosa* sp. nov. A, germination and elongation of hormogones within parent-sheath; B, two germinating hormogones; C and D, emergence of short germinating hormogones; E-G, emergence of long germinating hormogones, most of which are still enclosed within the parent-sheath; H and I, two germinating hormogones emerging side by side; J, filament with the outer sheath teased out to show the new sheath enclosing a long hormogone; K, two hormogones, enclosed within thick coloured sheaths, joined together by a small remnant of the parent-sheath (BHARADWAJA) . . . . . 141
1. Diagram showing degree of significance of difference between pairs of strains in early Bramley's Seedling apple, 1927 (DAS GUPTA) . . . . . 202
2. Diagram showing degree of significance of difference between pairs of strains in late Bramley's Seedling apple, 1927 (DAS GUPTA) . . . . . 203
3. Diagram showing degree of significance of difference between pairs of strains in Bramley's Seedling apple, 1928 (DAS GUPTA) . . . . . 207
4. Diagram showing degree of significance of difference between pairs of strains in Bramley's Seedling apple, 1929 (DAS GUPTA) . . . . . 210
5. Diagram showing degree of significance of difference between pairs of strains in Worcester Pearmain apple, 1929 (DAS GUPTA) . . . . . 210

## FIGURES.

PAGE

6.	Diagram showing rate of radial advance of strains of <i>Cytosporina ludibunda</i> in Bramley's Seedling and Worcester Pearmain apple, 1929 (DAS GUPTA) . . .	111
7.	Diagrammatic representation of the descent of strains of <i>C. ludibunda</i> (DAS GUPTA) . . .	213
8.	<i>Cytosporina ludibunda</i> . Diagram indicating relative pathogenicity of the saltants compared with that of the parent <i>C</i> (DAS GUPTA) . . .	213
9.	Diagram showing rate of radial advance of strain <i>C. ludibunda</i> in early and late Bramley's Seedling apple at 12° C., 1927 (DAS GUPTA) . . .	217
10.	Diagram showing degree of significance with age between pairs of samples of Bramley's Seedling apple inoculated with strain CA <sub>2</sub> , 1929 (DAS GUPTA) . . .	219
11.	Diagram showing degree of significance with age between pairs of samples of Bramley's Seedling apple, inoculated with strain CA <sub>4</sub> , 1929 (DAS GUPTA) . . .	220
12.	Diagram showing degree of significance with age between pairs of samples of Bramley's Seedling apple, inoculated with MK, 1929 (DAS GUPTA) . . .	221
1.	Number of chromosomes observed in 200 metaphases from the second division in the triploid compared with the expected in a triploid in which the extra set was distributed at random (SKOVSTED) . . .	239
1.	Balls composed of broken filaments of <i>Halopteris funicularis</i> (Mont.) Sauv. (DICKINSON) . . .	256
2.	Portion of a sporangial group of <i>Halopteris funicularis</i> from one of the Tasmanian balls showing sporangia and paraphyses (DICKINSON) . . .	257
3.	Section of a ball of <i>Halopteris funicularis</i> (DICKINSON) . . .	258
1.	<i>P. flavescens</i> var. <i>macrophyllum</i> . Diagram through a portion of the inflorescence apex, showing a young flower under a bract (BILLINGS) . . .	262
2.	<i>P. flavescens</i> var. <i>macrophyllum</i> . Primordia of carpels and placenta (BILLINGS) . . .	262
3.	<i>P. villosum</i> . Longitudinal section through flower with very young placenta, showing origin of the two nucellus-like projections (BILLINGS) . . .	263
4.	<i>P. flavescens</i> var. <i>macrophyllum</i> . Nucelli containing young archesporial cells (BILLINGS) . . .	264
5.	<i>P. villosum</i> . Longitudinal section of placenta (BILLINGS). . .	265
6.	<i>P. villosum</i> . Cross-section of placenta at archesporial cell stage (BILLINGS) . . .	265
7.	<i>P. villosum</i> . Heterotypic division of the embryo-sac mother-cell (BILLINGS) . . .	266
8.	<i>P. villosum</i> . Placenta in longitudinal section, showing the two embryo-sacs contained within it, both in the four-celled stage (BILLINGS). . .	266
9.	<i>P. villosum</i> . Intra-placental embryo-sac with four nuclei, two of which are about to enter a young haustorium-like long arm (BILLINGS) . . .	267
10.	<i>P. flavescens</i> var. <i>macrophyllum</i> . Long arm of embryo-sac with micropylar and chalazal nuclei just before division (BILLINGS) . . .	268
11.	<i>P. flavescens</i> var. <i>macrophyllum</i> . Placenta and embryo-sacs (BILLINGS) . . .	269
12.	<i>P. flavescens</i> var. <i>macrophyllum</i> . Egg apparatus and 'polars' (BILLINGS) . . .	270
13.	<i>P. flavescens</i> var. <i>macrophyllum</i> . Egg apparatus (BILLINGS) . . .	270
14.	<i>P. flavescens</i> var. <i>macrophyllum</i> . Double fertilization with a triple fusion (BILLINGS) . . .	270
15.	<i>P. flavescens</i> var. <i>macrophyllum</i> . Diagram of a longitudinal section of flower, showing the two embryo-sacs about the time of fertilization (BILLINGS) . . .	271
16.	<i>P. villosum</i> . Cross-section of placenta which contained short arms (BILLINGS) . . .	272
17.	<i>P. villosum</i> . Egg apparatus and young endosperm (BILLINGS) . . .	275
18.	Endosperm tissue in the base of the long arm of the embryo-sac (BILLINGS) . . .	275
19.	<i>P. flavescens</i> var. <i>macrophyllum</i> . Endosperm in expanded part only of the long arm (BILLINGS) . . .	276
20.	<i>P. villosum</i> . Endosperm in both long and short arms (BILLINGS) . . .	276
1.	The average potassium content in centigrams of leaves, axillary buds and stem segments at different levels of the haulm (JAMES and PENSTON) . . .	283
2.	The average $\frac{\text{potassium} \times 100}{\text{dry weight}}$ ratio for leaves, axillary buds and stem segments at different levels of the haulm (JAMES and PENSTON) . . .	286

FIGURES.	PAGE
3. Centigrams of potassium in the segments (leaf + bud + internode) up the haulm and its main axillary shoot (JAMES and PENSTON) . . . . .	286
4. The average content of potassium per segment expressed as weight per segment, percentage of dry weight, and percentage of water weight (JAMES and PENSTON) . . . . .	287
5. Diagram of the amount of potassium relative to total dry matter in a potato haulm during active growth (JAMES and PENSTON) . . . . .	288
6. Distribution between leaves of water and potassium relative to their dry weight, on three occasions of sampling (JAMES and PENSTON) . . . . .	289
7. As Fig. 6 showing the distributions in stems (JAMES and PENSTON) . . . . .	291
1. Graph showing the intensities of the different radiations in the two lights as measured by the micro-thermopile (DASTUR and SAMANT) . . . . .	303
1. A diagram made by plotting the planes of section of the roots in the slides labelled R.H.C., C.N., 269, 1-15. It demonstrates that the roots may grow up- or downwards (BENSON) . . . . .	314
1 A. A map of the stem in slide R.H.C., C.N., 269, 8. It indicates the close proximity in this case of the points of emission of the two roots shown in Text-fig. 1 and the crowded mass of rootlets just outside the stem (BENSON) . . . . .	314
2. A diagram made by plotting the planes of section of the roots in the slides R.H.C., C.N., 183 A-K (BENSON) . . . . .	314
1. <i>Paradoxopteris stromeri</i> Hirmer. Longitudinal sections. A. Tracheide with tyloses. B. Rectangular pericycle cells; endodermis; sclerenchyma sheath (EDWARDS) . . . . .	322
2. <i>Paradoxopteris stromeri</i> Hirmer. Transverse section of part of a bundle (EDWARDS) . . . . .	323
1. Loss in weight of <i>Pelvetia canaliculata</i> during inter-tidal exposure (ISAAC) . . . . .	345
1. Germinating zoospores, 2 days old (LEVYNS) . . . . .	350
2. Gametophyte, 17 days old (LEVYNS) . . . . .	350
3. Male gametophyte with two antheridia from one of which the spermatozoid has escaped (LEVYNS) . . . . .	351
4. Gametophyte with antheridia (LEVYNS) . . . . .	351
5. A small gametophyte bearing antheridia (LEVYNS) . . . . .	351
6. Spermatozoid (LEVYNS) . . . . .	351
7. Oogonium showing fertilization (LEVYNS) . . . . .	351
8. Zygote escaping from the oogonium (LEVYNS) . . . . .	351
9. Gametophyte with two old oogonia from which the young sporophytes have been extruded (LEVYNS) . . . . .	351
1. a. A normal leaf of <i>Oxalis Herrerae</i> . b. A leaf with small leaflets and swollen petiole. c and d. Swollen petioles from which leaflets have fallen (METCALFE) . . . . .	356
2. a. Transverse section through middle of an unswollen petiole of <i>O. Herrerae</i> . b. Transverse section of a swollen petiole (METCALFE) . . . . .	356
3. Transverse section showing peripheral tissues in an unswollen petiole of <i>O. Herrerae</i> (METCALFE) . . . . .	357
4. Peripheral tissues of a swollen petiole of <i>O. Herrerae</i> (METCALFE) . . . . .	357
5. Epidermal cells and three stomata from a swollen petiole of <i>O. Herrerae</i> (METCALFE) . . . . .	358
6. Transverse section of leaflet of <i>O. Herrerae</i> (METCALFE) . . . . .	358
7. Diagram of transverse section of part of a petiole of <i>O. bupleurifolia</i> (METCALFE) . . . . .	359
1. Separation of leaf-trace from reparatory strand, leaf-trace, adjoining the secondary wood (SCOTT) . . . . .	363
2. A. More advanced stage of separation, from another trace. B. Diagrammatic transverse section from upper end of old specimen (SCOTT) . . . . .	365
3. Leaf-trace far out in the wood, with a thick zone of its own secondary xylem (SCOTT) . . . . .	366
4. Circum-medullary strands giving off medullary strands (SCOTT) . . . . .	367
5. Tangential section of secondary wood, showing the medullary rays (SCOTT) . . . . .	371

FIGURES.	PAGE
1-3. Ascogenous hyphae of <i>Pyronema confluens</i> , showing intercalary cells from which the asci arise (GWYNNE-VAUGHAN and WILLIAMSON) . . . . .	378
4. Young hair of <i>L. scutellata</i> developing from intercalary cell of sheath hypha (GWYNNE-VAUGHAN and WILLIAMSON) . . . . .	379
5. Part of older hair of <i>L. scutellata</i> from sub-terminal cell (GWYNNE-VAUGHAN and WILLIAMSON) . . . . .	379
6. Three hairs of <i>L. scutellata</i> arising from adjacent cells (GWYNNE-VAUGHAN and WILLIAMSON) . . . . .	379
7. Part of trifurcate hair of <i>L. scutellata</i> (GWYNNE-VAUGHAN and WILLIAMSON) . . . . .	379
8. Hair from the sheath of the ascocarp of <i>L. stercorea</i> (GWYNNE-VAUGHAN and WILLIAMSON) . . . . .	379
1. Diagram showing degree of significance of difference between pairs of species of <i>Phomopsis</i> in Bramley's Seedling apples (DAS GUPTA) . . . . .	389
2. Diagram showing degree of significance of difference between pairs of species of <i>Diaporthe</i> in Bramley's Seedling apples (DAS GUPTA) . . . . .	391
3. Diagram showing degree of significance of difference between pairs of strains of <i>Diaporthe perniciosa</i> in Bramley's Seedling apples (DAS GUPTA) . . . . .	393
4. Diagram showing degree of significance of difference between <i>Cytosporina ludibunda</i> (parent and saltants) and species of <i>Phomopsis</i> and <i>Diaporthe</i> in Bramley's Seedling apples (DAS GUPTA) . . . . .	397
5. Diagram showing degree of significance of difference between <i>Cytosporina ludibunda</i> (parent and saltants) and species of <i>Phomopsis</i> and <i>Diaporthe</i> in Worcester Pearmain apples (DAS GUPTA) . . . . .	399
1-20. 1. Portion of the gametophyte of <i>Brachythecium rutabulum</i> showing the arrangement and divergence of the leaves. 2. Transverse section of the stem of <i>B. rutabulum</i> . 3. Transverse section of the leaf of <i>B. rutabulum</i> . 4. Longitudinal section of the stem of <i>B. rutabulum</i> . 5. (a) Portion of the gametophyte of <i>Philonotis fontana</i> showing the arrangement and divergence of the leaves. (b) Basal portion of the stem of <i>P. fontana</i> clothed with tomentum. (c) Portion of the tomentum. 6. Transverse section of the stem of <i>P. fontana</i> showing thin-walled epidermis and thickened hypodermis. 7. Transverse section of the leaf of <i>P. fontana</i> . 8. Longitudinal section of the stem of <i>P. fontana</i> . 9. Portion of the gametophyte of <i>Hypnum cuspidatum</i> showing the arrangement and divergence of the leaves. 10. Transverse section of a young stem of <i>H. cuspidatum</i> showing the thin-walled peripheral tissues and central strand. 11. Transverse section of the leaf of <i>H. cuspidatum</i> taken near its point of insertion on the stem and showing the double midrib. 12. Longitudinal section of the stem of <i>H. cuspidatum</i> . 13. Longitudinal section of the peripheral tissues of the stem of <i>H. cuspidatum</i> showing the thin-walled epidermis and pitted hypodermis. 14. Portion of the gametophyte of <i>Aulacomnium palustre</i> showing the arrangement and divergence of the leaves. 15. Transverse section of the stem and leaf of <i>A. palustre</i> . 16. Longitudinal section of the stem of <i>A. palustre</i> . 17. Portion of the gametophyte of <i>Campylopus brevipilus</i> showing the arrangement and form of the leaves. 18. Transverse section of the stem of <i>C. brevipilus</i> . 19. Transverse section of the leaf of <i>C. brevipilus</i> . 20. Longitudinal section of the stem of <i>C. brevipilus</i> (BOWEN) . . . . .	406
1-4. 1. Portion of a frond of <i>Athyrium pectinatum</i> , Wall. with <i>Cuscuta reflexa</i> , Roxb., wound tenaciously round the rachis of the fern in a clock-wise manner. 2. A section through the rachis with the parasite attached to it. The haustorium is seen to be arising centrally from the saddle-shaped structure and penetrating the rachis of the host. 3. Same as Fig. 2; showing the course of the haustorium through the cortex down to the vascular strand. The parasite and the forking of its haustorium is clearly seen. 4. A portion of Fig. 3 magnified to show the forking of the haustorium more clearly (SINGH) . . . . .	424
1. Photograph of the tank as seen from above (SINGH) . . . . .	426

FIGURES.	PAGE
2. Photograph of the tank on the microtome (SINGH) . . . . .	426
1. Diagrammatic representation of apparatus for regulating gas flow (GREGORY) . . . . .	427
1. Graph illustrating tiller development throughout life of variously treated plants (WARINGTON) . . . . .	443
1. Reconstruction of the stele of a cone of <i>E. arvense</i> L. (BROWNE) . . . . .	462
2. Reconstruction of the lower part of the stele of a cone of <i>E. maximum</i> Lam. (BROWNE) . . . . .	463
2 a. Reconstruction of the upper part of the stele of <i>E. maximum</i> Lam., of which Fig. 2 represents the lower part (BROWNE) . . . . .	464
3. Reconstruction of the stele of <i>E. palustre</i> (BROWNE) . . . . .	465
4. Reconstruction of the stele of <i>E. hyemale</i> (BROWNE) . . . . .	465
5. Reconstruction of the stele of cone of <i>E. limosum</i> (BROWNE) . . . . .	466
6. Reconstruction of the stele of cone of <i>E. giganteum</i> (BROWNE) . . . . .	466
7. Reconstruction of the stele of cone of <i>E. variegatum</i> (BROWNE) . . . . .	466
1. Diagram of $S_1/B_1$ (JANE) . . . . .	478
2. $S_1/B_1/1$ . A. Gynaeceum. B. Style and stigmas from above. C. Diagrammatic T.S. Pedicel (JANE) . . . . .	478
3. Floral Diagrams of: A. $B/1$ . B. $B/3$ (JANE) . . . . .	480
4. A-C. Corolla of $S_2/B_1/3$ . A. Right-hand side of corolla from outside, showing normal cleft between right antero-lateral and right postero-lateral petals. B. Corolla from left-hand side, showing deep cleft between left antero-lateral and left postero-lateral petals, and normal cleft between right antero-lateral and right postero-lateral petals. C. Left postero-lateral stamen. D. Flower $B/4$ , showing deep cleft between left antero-lateral and left postero-lateral petals (JANE) . . . . .	481
5. Flower $B/3$ (JANE) . . . . .	483
6. Stamens selected from various flowers, to show variation in the staminal appendages (JANE) . . . . .	484
7. Stamens of $S_1/17$ , a small bud about 5 mm. long (JANE) . . . . .	485
8. Stamens selected from various flowers to illustrate unusual types (JANE) . . . . .	486
9. Staminodes selected from various flowers (JANE) . . . . .	490
10. Camera lucida drawings of transverse sections of ovaries (JANE) . . . . .	493
11. Abnormalities in gynaeceum (JANE) . . . . .	494
1. Graph to show relationship between temperature and geotropic presentation time and latent time at the stage of maximum sensitivity of the epicotyl of <i>Lathyrus odoratus</i> (HAWKER) . . . . .	505
2. Graph to show effect of temperature on geotropic presentation time during the development of the epicotyl of <i>L. odoratus</i> (HAWKER) . . . . .	506
3. (a) cells, containing statoliths, from the endodermis of the epicotyl of <i>L. odoratus</i> grown at 20° C. (b) cells, containing statoliths, from the epicotyl of <i>L. odoratus</i> grown at 5-10° C. (HAWKER) . . . . .	510
4. The four series shown graphically (HAWKER) . . . . .	511
1. Non-sexual reproduction of <i>Monoblepharis</i> and <i>Gonapodya</i> (SPARROW) . . . . .	520
2. Sexual reproduction of <i>M. polymorpha</i> (SPARROW) . . . . .	524
1. Illustrating rates of growth of <i>S. fructigena</i> on potato agar of different initial acidities (HALL) . . . . .	551
2. Illustrating rates of growth of <i>S. fructigena</i> on a synthetic medium adjusted to different initial acidities (HALL) . . . . .	551
3. Illustrating effect of pH of medium on growth and zonation (HALL) . . . . .	554
4. Illustrating the effect of concentration of the medium on growth and zonation (HALL) . . . . .	556
5. Illustrating effect of illumination and acidity on growth of <i>S. fructigena</i> on the synthetic medium (HALL) . . . . .	559
6. Illustrating effect of illumination and acidity on growth of <i>S. fructigena</i> on potato-extract agar (HALL) . . . . .	559
7. Diagrammatic radial vertical section illustrating formation of ridge and hollow in <i>S. fructigena</i> (HALL) . . . . .	564

FIGURES.	PAGE
8. Diagram illustrating formation of ridges in <i>S. fructigena</i> due to light exposures of different duration (HALL) . . . . .	564
9. Illustrating formation of ridges in <i>S. fructigena</i> when light exposures (of 30 minutes each) are made twice daily (HALL) . . . . .	565
10. Diagrammatic radial vertical section showing ridges and hollows and thickening of underlying mycelium in zoning culture of <i>S. fructigena</i> (HALL) . . . . .	565
11. Illustrating the formation of subsidiary ridges in <i>S. fructigena</i> (HALL) . . . . .	567
12. Illustrating the relation of each ridge to periods of illumination and the formation of one subsidiary ridge in <i>S. fructigena</i> (HALL) . . . . .	567
13. Illustrating formation of subsidiary ridges in <i>S. frutigena</i> after one 6-hour exposure to daylight (HALL) . . . . .	567
14. Illustrating the relation of zones to light-exposures in <i>F. fructigenum</i> (HALL) . . . . .	567
15. Illustrating the effect of age of culture on width of zones in <i>F. fructigenum</i> (HALL) . . . . .	568
16. Illustrating the effect of daily light exposures of 10 minutes' duration on <i>F. fructigenum</i> (HALL) . . . . .	568
17. Illustrating the relation of ridges and hollows to periods of illumination in <i>S. fructicola</i> (HALL) . . . . .	571
18. Diagrammatic representation of surface view of culture of <i>S. fructigena</i> grown from a marginal fringe (HALL) . . . . .	571
19. Illustrating the relation of zones to temperature changes in <i>S. fructigena</i> (HALL) . . . . .	573
20. Illustrating the relation of zones to temperature changes in <i>F. fructigenum</i> (HALL) . . . . .	573
21. Diagram illustrating marginal staling effect in the agar sectors (HALL) . . . . .	577
1. Artificially calcified <i>Coprinus</i> sporophores (EWART) . . . . .	580
2. <i>Collybia</i> sporophores impregnated with cellulose acetate (EWART) . . . . .	580
3. <i>Coprinus</i> impregnated with cellulose acetate (EWART) . . . . .	581
4. <i>Pleurotus</i> soaked in formalin and carbolic acid and dried (EWART) . . . . .	582
5. <i>Coprinus</i> impregnated with urotropin and dried (EWART) . . . . .	582
6. <i>Coprinus</i> impregnated with phenolurotropin (EWART) . . . . .	583
1. Hydrolysis curves of leaf, bark, and wood extracts (PHILLIS and MASON) . . . . .	595
2. Hydrolysis curves of leaf extracts of three mono- and five dicotyledons (PHILLIS and MASON) . . . . .	596
3. Hydrolysis curves of sucrose, maltose, and of darkened leaf extract (PHILLIS and MASON) . . . . .	599
4. Hourly variations in sugars (grm. per 100 leaves) of lamina and petiole (PHILLIS and MASON) . . . . .	602
5. Daily variations in sugars (grm. per 40 leaves) of lamina and petiole (PHILLIS and MASON) . . . . .	604
6. Effect of ringing and darkening on sugars (grm. per 50 leaves) of lamina (PHILLIS and MASON) . . . . .	606
7. Effect of ringing and darkening on sugars (grm. per 50 leaves) of petiole (PHILLIS and MASON) . . . . .	608
8. Hourly and daily variations in sucrose concentrations (grm. per 100 grms. water) of lamina and petiole (PHILLIS and MASON) . . . . .	612
9. Daily variations in sucrose concentrations (grm. per 100 c.c. sap) of mesophyll, vein, inner and outer petiolar bark (PHILLIS and MASON) . . . . .	613
10. Sucrose and reducing sugar concentrations (grm. per 100 c.c. sap) of mesophyll, vein, and inner petiolar bark and also sucrose concentrations of outer petiolar bark of normal and ringed leaves (PHILLIS and MASON) . . . . .	615
11 A. Morning and evening concentrations (grm. per 100 c.c. sap) of sucrose on four successive days in three regions along the primary vein and in the mesophyll, with lines of closest fit and extrapolated concentrations for fine veins (PHILLIS and MASON) . . . . .	620
11 B. Morning and evening concentrations (grm. per 100 c.c. sap) of reducing sugars on four successive days in three regions along the primary vein and in the mesophyll, with lines of closest fit and extrapolated concentrations for fine veins (PHILLIS and MASON) . . . . .	621



## FIGURES.

## PAGE

12. Regression lines of dry weight (per 50 leaves) on time for mesophyll and veins of illuminated and darkened leaves (PHILLIS and MASON) . . . . . 627
13. Concentrations (grm. per 100 c.c. sap) of total sugars in mesophyll, veins, inner and outer petiolar bark and wood of illuminated and darkened leaves (PHILLIS and MASON) . . . . . 628
- 1-21. 1. Portion of the gametophyte of *Eurhynchium striatum* showing the arrangement and divergence of the leaves. 2. Transverse section of the stem of *E. striatum*. 3. Transverse section of the leaf of *E. striatum*. 4. Transverse section of the stem of *E. striatum* cut in the region of a developing branch and showing the continuity of the thin-walled tissue of the stem and branch. 5. Longitudinal section of the stem of *E. striatum*. 6. Longitudinal section of the peripheral tissues of the stem of *E. striatum* showing the pitted hypodermis. 7a. Portion of the branching region of the gametophyte of *Porotrichum alopecurum* showing the arrangement and divergence of the leaves. b. Portion of the lower region of the stem of *P. alopecurum*. c. Complete gametophytic plant of *P. alopecurum*. 8. Transverse section of the stem and leaf of *P. alopecurum*. 9. Longitudinal section of the stem of *P. alopecurum*. 10. Portion of the gametophyte of *Hylocomium squarrosum* showing the arrangement and divergence of the leaves. 11. Transverse section of a young stem of *H. squarrosum*. 12. Transverse section of an older stem of *H. squarrosum* showing an extreme thickening of the cells of the cortex resulting in the obliteration of the lumen of some of the cells. 13. Transverse section of the leaf of *H. squarrosum*. 14. Portion of the gametophyte of *Brachythecium purum* showing the arrangement and divergence of the leaves. 15. Transverse section of a young stem of *B. purum*. 16. Transverse section of an older stem of *B. purum*. 17. Transverse section of the leaf of *B. purum*. 18. Portion of the gametophyte of *Hypnum Schreberi* showing the arrangement and divergence of the leaves. 19. Transverse section of the stem of *H. Schreberi*. 20. Transverse section of the leaf of *H. Schreberi*. 21. Longitudinal section of the stem of *H. Schreberi* (BOWEN) . . . . . 638
- 22-41. 22. Longitudinal section of the peripheral tissues of the stem of *Hypnum Schreberi* showing the sparsely pitted hypodermis. 23. Portion of the gametophyte of *Hypnum cupressiforme* showing the arrangement and divergence of the leaves. 24. Transverse section of the stem of *H. cupressiforme*. 25. Transverse section of the stem and leaf of *H. cupressiforme* cut near the point of insertion of the leaf on the stem. 26. Longitudinal section of the stem of *H. cupressiforme*. 27. Longitudinal section of the peripheral stem tissues of *H. cupressiforme* showing a large-lumened epidermal cell and pitted hypodermis. 28a. Portion of the apical regions of the gametophyte of *Climacium dendroides* showing the arrangement and divergence of the leaves. b. Portions of the lower regions of the stem of *C. dendroides*. c. Complete gametophytic plant of *C. dendroides*. 29. Transverse section of the stem of *C. dendroides*. 30. Transverse section of the leaf of *C. dendroides*. 31. Longitudinal section of the stem of *C. dendroides*. 32. Longitudinal section of the stem of *C. dendroides* cut at the point of origin of a branch and showing the pitted cortex. 33a. Portion of the gametophyte of *Thuidium tamariscinum* showing the arrangement and divergence of the leaves of the stem and branches. b. Paraphylls of the stem of *T. tamariscinum*. 34. Transverse section of the stem of *T. tamariscinum*. 35. Transverse section of the leaf of *T. tamariscinum*. 36. Longitudinal section of the stem of *T. tamariscinum* showing epidermis, hypodermis, paraphylls, and leaf insertion. 37. Portion of the gametophyte of *Mnium punctatum* showing the arrangement and divergence of the leaves. 38. Transverse section of a young stem of *M. punctatum*. 39. Transverse section of an older stem and leaf of *M. punctatum*. 40. Transverse section of the leaf of *M. punctatum*. 41. Longitudinal section of the stem of *M. punctatum* (BOWEN) . . . . . 650

FIGURES.	PAGE
1-2. 1. Method of demonstrating reduction of pressure in the intercellular spaces of a submerged leaf. 2. Apparatus to demonstrate reduction of pressure in a submerged leaf (SEN and BLACKMAN) . . . . .	668
1-2. 1. Diagram showing electric drying oven with pump and condensing apparatus. 2. Diagram of Joly balances to show enclosure within glass tubes (BOLAS and MELVILLE) . . . . .	675
3. Graph showing effect of mass of material on time of drying of stems and leaves (BOLAS and MELVILLE) . . . . .	676
4. Phyllometer, i.e., apparatus to determine leaf area (BOLAS and MELVILLE) . . . . .	677
5. Details of phyllometer shown in Fig. 4 (BOLAS and MELVILLE) . . . . .	678
6. Calibration curves of phyllometer for two observers (BOLAS and MELVILLE) . . . . .	679
7. Shaking apparatus for removal of earth from roots (BOLAS and MELVILLE) . . . . .	680
8. Seasonal drift of assimilation rate (BOLAS and MELVILLE) . . . . .	684
9-10. 9. Relationship to light of increase in dry weight of plant and leaf. 10. Relationship to light of fall of water-content of leaf and plant (BOLAS and MELVILLE) . . . . .	685
11. Relationship between assimilatory rate and fall in water-content (BOLAS and MELVILLE) . . . . .	685
12. Seasonal drift of water-content of whole plant and of leaf (BOLAS and MELVILLE) . . . . .	686
13. Graph showing relationship between leaf-area increase during a period of seven hours and the amount of light received (BOLAS and MELVILLE) . . . . .	687
14. Distribution of assimilatory material between leaf, stem, and root (BOLAS and MELVILLE) . . . . .	688
1. Map showing temperate South America (GOOD) . . . . .	695
2. The distribution of the genera Gaultheria and Pernettya as taken from the Kew Index (GOOD) . . . . .	713
1. Diagram showing proportion of singles according to the size of family (FISHER) . . . . .	729
1-24. For explanation see text (DICKSON) . . . . .	741
25. Graph showing diameters of different strains from the second to the eighth day on the medium containing 4.0 per cent. glucose (DICKSON) . . . . .	746
26. Graph showing mean increase in diameter per twenty-four hours growth for each strain plotted against the different concentrations of glucose (DICKSON) . . . . .	747
27. Graph showing relationship between concentration of asparagin and mean daily increase in diameter of the colony over the whole period during which measurements were made (DICKSON) . . . . .	749
1-12. <i>Olpidiopsis Ricciae</i> , nov. sp. 1. Young zoosporangia in the base and tube of a plain rhizoid. 2 and 3. Mature and empty zoosporangia and liberated zoospores in plain rhizoidal tubes. 4. Young sporangia in a peg rhizoid. 5. Oospores in a peg rhizoid. 6. Oospores in a plain rhizoid. 7 (a) Zoosporangium two and a half hours before spore ejection. (b) Same zoosporangium fifteen minutes before opening. 8. Various stages in oospore initiation. 9. Empty antheridial cell attached to a maturing oospore. 10 (a) and (b). Biciliate zoospores in stained and unstained sections. 11 (a) Zoospore in the act of penetration. (b) and (c). Germination of zoospores by germ-tubes. 12. Beak-like opening of zoosporangia cultured in tap-water (DU PLESSIS) . . . . .	758
1. Conidia of <i>Vorticillium Malthousei</i> sp. nov. from mushroom (WARE) . . . . .	779
2. Branching of conidiophores of <i>V. Malthousei</i> (WARE) . . . . .	779
3. Conidiophore of <i>V. Malthousei</i> (WARE) . . . . .	779
4. The mode of branching of conidiophores of <i>V. Malthousei</i> (WARE) . . . . .	781
5. The formation of the spore-masses of <i>V. Malthousei</i> at the tips of the secondary branches (WARE) . . . . .	783
6. Large and small spore-masses of <i>V. Malthousei</i> observed in process of dissociation (WARE) . . . . .	783
1-8. 1. Whole plant. 2. Cyst formation. 3. Germination of zoospore with commencing rhizoid. 4. Fusion of gametes; the two nuclei before fusion. 5. Germination of zygote. 6. Longitudinal division of mature specimen. 7. Vegetative multiplication. 8. Escape of non-motile spores (NAVAT) . . . . .	789

FIGURES.	PAGE
9-13. Swarmers. 9. Zoospore. 10. Facultative gamete. 11, 12, 13. Compound swarmers. 12. Shows division into two units (NAYAL) . . . . .	791
14-17. 14. Part of section of the aerial portion showing pyrenoids and nuclei. 15. Zoospore come to rest. 16. Zygote. 17. Formation of resting spores (NAYAL) . . . . .	792
1. Diagrammatic sagittal section through larva of <i>Rhopobota vacciniaria</i> (SAWYER) . . . . .	803
1. Twelve bivalents and a fragment at diakinesis in <i>Tulipa</i> (STONE) . . . . .	820
2. <i>a</i> , anaphase in <i>Tulipa</i> showing fragmentation and lagging bivalents. <i>b</i> , late anaphase. <i>c</i> , late second division anaphase showing lagging fragments and disintegrating chromatin (STONE) . . . . .	821
3. <i>a</i> , part of the ring of chromosomes at metaphase in <i>Rhoeo discolor</i> showing an abnormal bead of chromatin attached to the ends of two chromosomes. <i>b</i> , telophase of the first division showing lagging fragments (STONE) . . . . .	821
4. <i>a</i> , normal somatic metaphase of <i>Crocus Olivieri</i> . <i>b</i> , <i>c</i> , <i>d</i> , abnormal plates induced by treatment (STONE) . . . . .	822
1. Germination of indigenous cocksfoot (BcS 36, 14 months old) at 19° C. A. Unsoaked caryopses. B. Soaked caryopses. C. Unsoaked 'seeds' (caryopses with pales). D. Soaked 'seeds' (caryopses with pales) (CHIPPINDALE) . . . . .	844
1-8. 1. Plasmodium almost filling the host cell, and a younger one separated by a septum. 2. Plasmodium showing furrowing prior to the formation of zoosporangia. 3. Zoosporangia lying freely in the host filament. 4. Zoosporangium, showing the division of the cytoplasm into zoospores. 5. Zoosporangium in which the zoospores have been delimited. 6. Zoospores after liberation, showing the flagella directed backwards from the anterior end. 7. Empty zoosporangia showing the exit tube through which the zoospores have escaped. 8. Group of empty zoosporangia in host filament (IVIMEY COOK and NICHOLSON) . . . . .	854
9-16. 9. Early stage in infection by a zoospore showing the infection hypha penetrating the host wall. 10. Late stage in infection by a zoospore showing the almost empty zoospore and the formation of an amoeboid body within the host. 11. Young plasmodia shortly after the separation of the amoebae from the infection hyphae. 12-13. Mature cystosorus lying in the apical segment of a swollen hypha. 14. Mature cystosorus showing the elongated shape sometimes adopted. 15. Some resting spores showing the thickness of the wall. 16. Some spores after the escape of the spore, showing the small hole by which it escaped (IVIMEY COOK and NICHOLSON) . . . . .	856
1. A. Transverse section of an almost mature capsule with two well-developed seeds in each loculus. B. Transverse section of a young ovary showing four ovules, two in each loculus. C. Longitudinal section of a young ovary of a typical plant, showing two lower ovules and the upper ovule attached to the axile placenta. D. Placental tissue removed from the capsule lid to show the position of the upper seed. E. Capsule lid as shed from the plant, showing the remains of the flower and the placental tissue still in position. F. Upper seed. Dry. G. Same seed after soaking in water for ten minutes. H. One of the lower seeds from the same capsule as the upper seed shown in Fig. 1 K. Face view. J. The same seed in edge view. K. Upper seed, edge view. L. The same seed in face view. M. Lower seed dry. N. Same seed after soaking in water for ten minutes (DOWLING) . . . . .	862
2. Variation in length and breadth of upper and lower seeds (DOWLING) . . . . .	866
3. Germination of lower seeds. September (DOWLING) . . . . .	870
1. <i>Northea seychellana</i> Hook. fil. 1. A fruit showing the cap-like covering of the orifice of the seed cavity. 2. The emerging radicle, the cap or valve having been pushed away. 3. A later stage. 4. The radicle and the elongated hypocotyls have carried the plumule out of the cavity and it is now emerging from between the stalks of the cotyledons (HILL) . . . . .	874
2. <i>Prunus Cerasus</i> L. 1. The fruit in transverse section showing the line of cleavage of the stone. 2. Diagrammatic representation of the arrangement of the cells	

## FIGURES.

## PAGE

- on either side of the cleavage line. 3. A germinating fruit showing the stone splitting into two halves (HILL) . . . . . 875
3. *Nyssa sylvatica* Marsh. 1. The fruit. 2-5. The endocarp showing the valve or shutter in face or side view (2 and 3) and being pushed away by the emerging radicle (5). 4. Back view of the stone. 6. The valve detached. 7. The fruit in transverse section. 8 and 9. Seeds germinating with the valve pushed aside in 8 and completely thrown off in 9 (HILL) . . . . . 876
4. *Nyssa sylvatica* Marsh. A. A fossil fruit from the Pliocene deposits, Reuver. B. Fossil specimens from the Pliocene deposits, Swalmen, showing valves (HILL) . . . . . 877
5. *Cornus officinalis* Sieb. et Zucc. 1. The complete bilocular fruit showing the two valves commencing to split away. 2. A view of the apex showing the radicles of the two embryos pushing away the valves. 3. Side view with a valve completely removed exposing the seed. 4. The detached valve. 5. The fruit in transverse section with the two loculi (HILL) . . . . . 878
6. *Canarium Schweinfurthii*, Engl. 1. The stone with one of the valves indicated. 2. The same with the valve removed. 3. The valve or fenestra seen from the inside. 4. The stone in cross section showing three loculi, only one fertile and the fenestra in section. 5 and 6. Two stages in germination, the fenestra being lifted up by the radicle. 7. A stone with two empty loculi and the remains of the testas. 8. A seedling with its arched hypocotyl and the peg-like base of the hypocotyl pressing against the edge of the stone. 9. A similar picture showing the cotyledons being drawn out of the testa and the peg of the hypocotyl pressed against the edge of the cavity. 10. The peg seen from the front and from the side (HILL) . . . . . 879
7. *Tectona grandis* Linn. fil. 1. Fruit with outer coverings removed showing the four valves and the emergent radicle of one seedling. 2. The same from above to show the four valves and the ribs of the stony endocarp. 3. The skeletal core of the stone and one carved valve detached (HILL) . . . . . 880
8. *Haematostaphis Barteri* Hook. fil. 1. The stone after the removal of the fleshy pericarp showing the 'lid' or valve at the apex. 2. Apical view, showing the valve with its median groove. 3. The valve seen from the outside and from the inside. 4. The commencement of germination. 5. The valve removed showing the apex of the radicle lying in the cavity of the stony endocarp. 6. The stone in section showing the valve and the seed in the cavity (HILL) . . . . . 880
9. *Sclerocarya caffra* Sond. 1. The stone after removal of the fleshy outer coats of the fruit. 2. The same with one of the lids being lifted by the germinating seed. 3. Stone seen from above with two lids removed to show the position of the germinating embryo. 4. The stone in section showing the relation of the lid to the rest of the endocarp. 5. A young seedling, the cotyledons being drawn out of the cavity (HILL) . . . . . 881
10. *Dracontomelon mangiferum* Blume. 1. Surface view of the stone after removal of the fleshy pericarp and mesocarp showing the lids closing the cavities in the stone which contain the ovules. 2. The stone from the side with two lids in position. 3. A lid detached seen from the inside. 4. The lid from the side (HILL) . . . . . 882
11. *Pleiogynium Solandri* Engl. 1. The stone with outer covering removed, a fluted basin-like structure with a domed upper surface. 2. The same to show the orifices in the stony mesocarp with the apices of the endocarp caps within the cavities. 3. The fruit in transverse section. 4. The same in longitudinal section. 5. The cap or lid of the endocarp from the side, front and back (HILL) . . . . . 883
12. *Davidia involucrata* Baill. 1. The stone with one germinating seed. 2. A later stage in germination showing three seedlings and the cavities in which the embryos lie. 3. The valves, (c) from the side, (d) the exterior appearance with grooves continuous with the grooves on the lower part of the stone, (e) from the inside showing the concave inner surface, the pointed apex and

## FIGURES.

## PAGE

- the truncated base. 4. A stone in transverse section showing seven loculi, only two in this case fertile (HILL) . . . . . 885
- 1-23. 1. Portion of the gametophyte of *Hypnum cupressiforme* var. *filiforme* showing the arrangement and divergence of the leaves. 2. Transverse section of a branch of *H. cupressiforme* var. *filiforme* showing the absence of small-celled central tissue. 3. Longitudinal section of the stem of *H. cupressiforme* var. *filiforme*. 4. Portion of the gametophyte of *Dicranum scoparium* showing the arrangement and divergence of the leaves. 5. Transverse section of a young stem of *D. scoparium*. 6. Transverse section of the leaf of *D. scoparium*. 7. Longitudinal section of the stem of *D. scoparium*. 8. Portion of the gametophyte of *Hylocomium triquetrum* showing the arrangement and divergence of the leaves. 9. Transverse section of the stem and leaf of *H. triquetrum*. 10. Longitudinal section of the stem of *H. triquetrum*. 11. Portion of the gametophyte of *Ditrichum flexicauli* var. *densum* showing the arrangement and divergence of the leaves. 12. Transverse section of the stem and leaf of *Ditrichum flexicauli* var. *densum*. 13 *a*. Portion of the gametophyte of *Anomodon viticulosus* showing the normal arrangement and divergence of the leaves. 13 *b*. Portion of the gametophyte of *Anomodon viticulosus* showing the arrangement and divergence of the leaves in wilted forms. 14. Transverse section of the stem and leaf of *A. viticulosus*. 15. Longitudinal section of the stem of *A. viticulosus*. 16. Portion of the gametophyte of *Mnium hornum* showing the arrangement and divergence of the leaves. 17. Transverse section of the stem of *M. hornum*. 18. Transverse section of the leaf of *M. hornum*. 19. Longitudinal section of the peripheral tissues of the stem of *M. hornum* showing the pitted walls of the epidermis and hypodermis. 20. Portion of the gametophyte of *Mnium undulatum* showing the arrangement and divergence of the leaves. 21. Transverse section of the stem of *M. undulatum*. 22. Transverse section of the leaf of *M. undulatum*. 23. Longitudinal section of the stem of *M. undulatum* (BOWEN) . . . . . 892
1. *Droyoxylon*, sp. : (transverse section). Diagram illustrating the general type of structure of v. 23315 (BANCROFT) . . . . . 916
2. *Helictoxylon*, sp. (transverse sections). *a*. Diagram of the structure of v. 23313, showing an area of larger and more numerous vessels followed by one with fewer and smaller vessels. *b*. Diagram of the structure of v. 23314, showing the central portion of a vascular cord, with what appears to be central and periaxial secondary wood (BANCROFT) . . . . . 918



## Studies in Growth and Differentiation.

### IV. The Distribution of Some Solutes in the Tissues of *Kleinia articulata*.

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With three Figures in the Text.

IT has been shown in a previous paper (13) that in the stem of *Kleinia articulata*, growing in a greenhouse in ordinary soil, calcium and phosphate accumulate in different tissues. Calcium was found in the pith and parts of the inner cortex, largely in the form of calcium malate, while phosphate was practically confined to the bundle zone. The sharpness of the localization was particularly remarkable, especially in the case of calcium, and it was clearly desirable to obtain information regarding the occurrence and distribution of other solutes commonly occurring in the sap of plants.

It has not been possible in every case to precipitate a substance *in situ* and so determine with exactitude or in full detail its distribution in the tissues. Since, however, the pith, bundle zone, and cortex are in the *Kleinia* stem so well contrasted, useful information can be obtained by quantitative analysis after approximate separation of these zones with a scalpel. The possibilities of this procedure have already been indicated in relation to acidity and to calcium and phosphate contents, for which microchemical methods were also available for comparison. In the work to be described both methods were used as a check on one another when both were available. In one or two cases tests not capable of giving information regarding localization when applied to whole sections were made to yield useful results by applying them to small excised portions of sections of approximately equal size.

As the methods used may prove of service to other investigators they are collected together in the first part of the paper and the results are given in the second part.

The following substances were investigated :

Kations: potassium, sodium, magnesium, aluminium, iron, ammonium.

Anions: nitrate, sulphate, chloride, oxalate.

In addition the localization of inulin was determined, this substance being the chief storage carbohydrate. The localization of aluminium was deemed to be of especial interest, since this substance in the form of aluminium malate has been found by Hempel (7) to occur in the leaves of most succulents and to exert a considerable buffer action in the expressed juice.

Determinations of the alcohol-soluble reducing and non-reducing sugars showed relatively low concentrations for leaves and stems.<sup>1</sup> Attempts to localize reducing sugars by the osazone method were unsuccessful.

The emphasis by Ruhland and Wetzell and others of the important part played by deamination processes, leading to the production of ammonia, in acid-producing plants, made it desirable to obtain an estimate of the ammonia content.

We take this opportunity of conveying our best thanks to Dr. A. E. Bradfield of the Dept. of Chemistry in this College, and also to Dr. W. O. Jones, who at various times kindly discussed the applicability of some of the chemical methods and offered fruitful suggestions and friendly criticism. We are also indebted to Mr. N. Woodhead for providing material of *K. articulata* in the quantity necessary for this investigation.

## PART I. METHODS.

### *Potassium.*

(a) *Microscopical.* Two reagents were tried. (Cf. Molisch (8).)

(1) A freshly made solution of sodium cobaltinitrite in 10 per cent. acetic acid. (2) A 10 per cent. solution of platinic chloride (a) in water and (b) in 70 per cent. alcohol. With the first of these the precipitate

<sup>1</sup> The following results may be of interest :

Material collected 2.30 p.m. Oct. 24, 1930. Leaves slightly wilted.

Dry weight: leaves 6.83 per cent., stems 6.10 per cent.

Leaves.	{ Reducing sugars . . .	0.054	per cent. fresh wt.	0.80	per cent. dry wt.
	{ Non-reducing sugars . . .	0.082	" "	1.20	" "
	{ Total . . . . .	0.136	" "	2.00	" "
Stems.	{ Reducing sugars . . .	0.112	" "	1.82	" "
	{ Non-reducing sugars . . .	0.029	" "	0.47	" "
	{ Total . . . . .	0.141	" "	2.29	" "



often took some time to come down, owing probably to slow penetration of the reagent into the cells. A similar result is obtained with an aqueous solution of platinic chloride, but a solution in 70 per cent. alcohol penetrates immediately, and precipitation, though not instantaneous, is more rapid.

It was estimated that precipitation was complete in about one minute. This may be long enough to allow of some slight diffusion of potassium from the cells before precipitation. In view, however, of the degree of uniformity of the results when individual specimens are compared, it is unlikely that this source of error is of considerable magnitude, though it may account for occasional small quantities of potassium in zones which are more often quite devoid of potassium.<sup>1</sup>

(b) *Quantitative*. For the quantitative determinations plants were dried in an oven at 95° C., and a definite weight was incinerated to ash. The potassium was determined by the well-known perchlorate method (see Cumming and Kay (5)).

#### *Sodium.*

(a) *Microchemical*. No method has yet been devised for the precipitation of sodium inside the cells. The microchemical method recommended by Molisch involves the formation of the characteristic crystals of sodium uranyl acetate on the slide outside the section, and it is not in our experience highly satisfactory even for the demonstration of its presence. An attempt to precipitate sodium in the cells as sodium cobalt uranyl acetate was unsuccessful.

The distribution of sodium was therefore investigated by separation of the zones of the stem with a scalpel, and the application of quantitative methods to the separated tissues.

(b) *Quantitative*. The sodium was determined by a new method due to Caley and Foulk (3). It involves the precipitation of sodium as sodium magnesium uranyl acetate having the formula  $\text{NaC}_2\text{H}_3\text{O}_2 \cdot \text{Mg}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{UO}_2(\text{C}_2\text{H}_3\text{O}_2) \cdot 6(\frac{1}{2}\text{H}_2\text{O})$ . As the weight of sodium is only 0.0153 times the weight of the precipitate the method may be used for very small amounts of sodium.

The method is carried out on the ash, and the procedure adopted was as follows:

<sup>1</sup> Patschowsky (9), in connexion with his comprehensive study of the occurrence of oxalic acid and soluble oxalates in plants, showed that precipitation occurs inside the cells if the reagent is in greater concentration outside than the equivalent of the concentration of the reactant inside. Our use of alcoholic reagents ensures rapid entry into intercellular spaces, and maximum permeability by killing the protoplasm. If the reagent is strong enough, localization should be very sharp, as the reagent is applied simultaneously to both sides of a section about  $\frac{1}{2}$  mm. thick. The chances of lateral diffusion are minimal, and unlikely to be appreciable unless precipitation is slow, when there should also be some precipitation outside the section. A solution of 10 per cent. platinic chloride in 70 per cent. alcohol was finally adopted for most of the observations, although concordant results were obtained with the cobaltinitrite reagent.

The ash is taken up in dilute HCl, filtered to remove silica and any other insoluble substances, the calcium phosphate present precipitated by the addition of ammonium chloride and ammonia and filtered off. The phosphate-free solution is then neutralized, and 5 c.c. taken for the sodium determination. About 100 c.c. of the reagent (see Caley and Foulk, *loc.*) is added for every 10 mg. or less of sodium, and the beaker containing the mixture is placed in a bath at 20° C., and vigorously stirred for 30 to 45 minutes. In the present instance the stirring was worked by a water-turbine. The precipitate is filtered through a weighed Gooch crucible, washed with 5 c.c. portions of 95 per cent. alcohol, dried at 105° C. for 30 minutes, cooled, and weighed.

### *Magnesium.*

(a) *Microchemical.* The method of Richter was employed (Molisch (8)). Sections are quickly rinsed and dried with blotting paper, and placed in a 0.1 per cent. solution of sodium ammonium phosphate ( $\text{NaH}\text{NH}_4\text{PO}_4 \cdot 12\text{H}_2\text{O}$ ) in a watch glass. This is then placed for one minute in an atmosphere of ammonia (e.g. the watch glass can be supported on a glass tripod in a crystallizing dish containing strong ammonia over which another dish is inverted). Magnesium is precipitated as magnesium ammonium phosphate, the characteristic crystals of which can be recognized and their distribution followed after removal of air from the tissues under reduced pressure.

(b) *Quantitative.* The plants were dried in an oven, and a weighed quantity of dry matter reduced to ash. After incineration the ash was digested with hot dilute HCl, filtered, and phosphates and hydroxides precipitated by the addition of ammonium chloride and ammonia. The filtrate was boiled to expel excess ammonia and, while hot, excess of ammonium oxalate added to remove the calcium, and the precipitate of calcium oxalate filtered off. A few drops of methyl orange are added to the filtrate, which is then neutralized to an orange tint with HCl and boiled. To the boiling solution is added excess of a 5 per cent. solution of microcosmic salt (sodium ammonium phosphate) and the solution allowed to cool. When cool, excess of strong ammonia is added and the solution stirred until precipitation is complete. The precipitate is allowed to stand for 24 hours, filtered off through analytical filter paper, and well washed with dilute ammonia solution, the latter being drained off the filter paper as completely as possible. The precipitate is now dissolved in the least possible quantity of dilute HCl, a few drops of microcosmic salt solution added, and the solution again made alkaline with strong ammonia, stirred until precipitation is complete, and allowed to stand for a few hours. The precipitate is filtered off, heated to dull redness, and weighed as magnesium pyrophosphate ( $\text{Mg}_2\text{P}_2\text{O}_7$ ).

*Aluminium.*

(a) *Microchemical.* The precipitation of aluminium as caesium alum advocated by Molisch (Kratzmann's modification of Behren's original method), though quite suitable for solutions of aluminium containing 0.2 per cent. or over, was not successful with the much lower concentrations found in the cell sap of *Kleimia*. Attempts to precipitate aluminium with ammonium fluoride as ammonium aluminium fluoride were also unsuccessful. The localization of aluminium, like that of sodium, has therefore been obtained by separation of the tissues, and the quantitative determination of aluminium in the separated zones.

(b) *Quantitative.* The presence of aluminium in small quantities was demonstrated by a chemical analysis of the ash. The quantitative estimation of aluminium in plants is a matter of considerable difficulty, mainly because of the interference of other substances, especially calcium phosphate and iron. It is, indeed, doubtful whether most of the aluminium determinations in plants are reliable. In the present work the aluminium was determined by two separate methods.

The first of these is the ordinary chemical method in which the aluminium is separated from the phosphates and other interfering substances by the ordinary procedure and weighed as aluminium oxide. This method was long and tiresome and, owing to the relatively small quantity of aluminium present, with resulting incomplete precipitation, errors of weighing, &c., it was not very satisfactory.

The method used in most of the work was a modification of the colorimetric method, which has recently been critically investigated by Yoe and Hill (14) and found to be very satisfactory. The method depends on the formation of a 'lake' when aluminium is precipitated in the presence of the dye alizarin-red monosulphonate (alizarin red S).

The analysis was carried out on the ash, which is dissolved in hot, fairly strong HCl, the solution filtered, and phosphates and hydroxides precipitated with ammonium chloride and ammonia. The precipitate is filtered off and digested with hot NaOH, which dissolves the aluminium hydroxide, but leaves any ferric hydroxide and calcium phosphate behind. The NaOH solution, containing the aluminium as sodium aluminate, is neutralized with strong HCl until the aluminium begins to be precipitated; just sufficient dilute HCl is then added to redissolve the aluminium. An aliquot part of this solution is used for the colorimetric determination of aluminium as described by Yoe and Hill (*loc. cit.*). The presence of the very small quantities of iron in *K. articulata* was sufficient to make the method inapplicable unless iron was first removed, as by the treatment described above.

Using standard solutions of aluminium, the method gave much more accurate results than the ordinary quantitative chemical method, with the small concentrations present in the material under investigation.

### *Iron.*

The amount of iron present was so small that no attempt was made to estimate it quantitatively. Qualitative data have, however, been obtained incidentally in the course of aluminium estimations. When the 'Group III' precipitate (calcium phosphate and hydroxides of iron and aluminium) was digested with hot NaOH to dissolve the aluminium, the presence after filtration of brown ferric hydroxide on the filter paper was easily discerned in the case of leaves, but not with stems. The precipitate was in all cases redissolved in HCl and tested with potassium ferrocyanide: the intensity of the blue colour gave clear indications of the relative amounts of iron present.

### *Ammonium.*

*Quantitative.* Ammonia present as ammonium salts was determined by the aspiration method as described by Sessions and Shive (11). The analysis is carried out on the extracted sap, to which is added a saturated solution of sodium carbonate containing sodium chloride. The ammonia is aspirated off at ordinary temperatures and absorbed in standard sulphuric acid. The aspiration was continued for 12 hours. A number of determinations can be carried out together, using the same current of air.

### *Nitrate.*

(a) *Microchemical.* A close indication of the distribution of nitrate was obtained by the use of the diphenylamine reaction on the separated tissues.

(b) *Quantitative.* Two methods were applied to the quantitative analysis. The first is an aspiration method as described by Sessions and Shive (11). Devarda's alloy finely ground to a powder in dilute alkaline solution is used for the reduction of nitrate to ammonia, which is aspirated off and absorbed in standard acid as described for the estimation of ammonia.

The second is the ordinary colorimetric method with phenyl sulphonic acid.

The analyses by both methods were carried out on the extracted sap, and the results agreed very well. The colorimetric method is much quicker than the aspiration method, and was used in most of the later determinations. The large quantity of nitrate present made the use of greatly diluted extracts possible, and it was therefore found unnecessary to clarify the extract before the determination.

*Sulphate.*

Sulphate determinations were carried out on the extracted sap, which was first cleared by boiling with 1 c.c. of acetic acid for every 50 c.c. of sap. The precipitated proteins, &c., which were small in quantity, were filtered off, and the sulphate precipitated as barium sulphate, as in ordinary chemical analysis.

*Chloride.*

The chloride determinations were carried out on the ash, which was taken up in dilute nitric acid and the chloride precipitated as silver chloride in the dark.

## PART II. RESULTS.

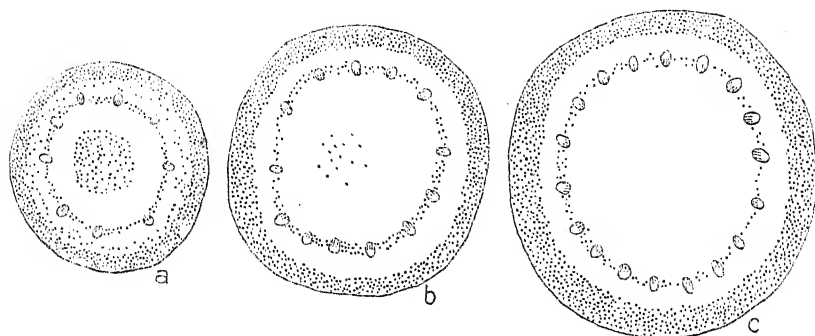
*Potassium.*

FIG. 1. Distribution of potassium (dotted) in transverse sections of different parts of the same *Kleinia* plant: (a) very young branch; (b) rather older branch, near apex; (c) same, near base.

(a) *Microchemical.* Plants grown in ordinary soil were rather poor in potassium, which was nearly confined to the outer cortex. There was none in the pith and only a few stray crystals in the bundle zone. Much more was present in plants grown in sand culture and watered weekly with a complete nutrient solution (Shive's normal). Here, again, it was in greatest quantity in the outer cortex. Some plants showed potassium in the centre of the pith. The differences depend in part on the age of the stem. Fig. 1 illustrates this. In a very young joint or near the apex of a growing shoot potassium occurred in a circular area in the middle of the pith as well as in the outer cortex and to a small extent in the bundle zone. In older parts the potassium in the pith diminished in amount and finally disappeared altogether. This is accompanied by increased accumulation in the outer cortex. Underground rhizomes were not generally as rich in

potassium as the aerial stem: it was found only in the outer cortex, except that in many specimens it showed a peculiar aggregation in the epidermis. (As no periderm was observed the rhizomes were probably quite young.)

In the leaf, potassium was found in the water-storage tissue only, increasing in amount up to middle age and disappearing in old leaves.

(b) *Quantitative.* The results of quantitative analyses are summarized in Table I.

TABLE I.

Per 100 grm. fresh weight.				
		Dry weight.	Potassium.	
		grm.	grm.	mg. equivalents.
Dec. 3, 1930; 4 p.m.				
Soil	{ Young joints	3.52	0.302	7.74
	{ Old joints	5.00	0.22	5.64
Sand culture (normal)	{ Young joints	3.53	0.171	4.40
	{ Old joints	5.85	0.285	7.31
Dec. 1, 1930; 7.30 p.m.				
Plants grown in sand culture.				
Young joints	Leaves	—	0.196	5.02
	{ Cortex	6.31	0.403	10.33
	{ Bundle zone	3.93	0.228	5.85
	{ Pith	2.12	0.138	3.54
Old joints	{ Cortex	9.65	0.451	11.6
	{ Bundle zone	4.46	0.281	7.2
	{ Pith	2.51	0.088	2.2

They are in accord with the microchemical observations. The cortex shows the highest concentration of potassium, the pith the lowest. The data for whole joints are somewhat irregular: although about five joints were used for each of the determinations, no stress can be laid on the different relation between the potassium content of young and old joints in soil and in sand culture respectively. Sand-culture plants of December 3 and December 1 do, however, both show more potassium in the old joints.

### *Sodium.*

A rough idea of the localization of sodium was obtained by separating the cortex, bundle zone, and pith, drying in an oven, dissolving the ash from equal weights of dry matter, and testing with cobalt uranyl acetate, which gives a heavy yellow precipitate consisting of characteristic crystals of sodium cobalt uranyl acetate. The quantity of precipitate showed that the bulk of the sodium is in the cortex, relatively little in the pith, and an intermediate amount in the bundle zone.

Some quantitative results are given in Table II. They confirm the

distribution indicated by the rough test. This distribution is very similar to that of potassium.

TABLE II.

		Per 100 grm. fresh weight.		
		Dry weight. gram.	Sodium.	
			gram.	mg. equivalent.
Dec. 9, 1930.				
Soil	{ Leaves	2.53	0.130	5.6
	{ Stems	3.20	0.152	6.6
Sand culture	{ Leaves	3.39	0.161	7.0
	{ Stems	3.66	0.132	5.7
Dec. 15, 1930.				
Soil (mature joints)	{ Cortex	7.4	0.205	9.0
	{ Bundle zone	3.61	0.159	6.9
	{ Pith	1.93	0.078	3.4

The sodium content is of the same order in sand culture as in soil. This was surprising, as no sodium was included in the culture solution added to the sand. Sand from the same source has since been found to contain sodium chloride in appreciable amounts. Some of this probably remained, notwithstanding the preliminary washing which the sand received before use.

### Magnesium.

(a) *Microchemical.* The stem is not rich in magnesium. A central patch of the pith contains most. The outer pith where calcium is most abundant is much poorer in, but not devoid of, magnesium. The perimedullary region where calcium is absent contains a small quantity, in some cells at random. There are minute traces in the inner cortex, again at random in some of the cells. In the bundle zone proper there is little if any. The outer cortex also appears to be devoid of magnesium (i.e. inorganic magnesium or magnesium ions).

(b) *Quantitative* (Table III). Analysis of whole organs showed accumulation of magnesium in stems with age, and least magnesium in the leaves.

There is no great difference in magnesium content between the different zones on a basis of fresh weight, but on a dry weight basis the pith has the highest percentage. It has also to be remembered that the poor outer pith and richer inner pith have been averaged together and that the results represent total magnesium. Thus in the cortex the magnesium content of the chlorophyll is included. The quantity found in the bundle zone is larger than would be expected in view of the microchemical observations and it is possible that it may be present here also in some combined organic form.

TABLE III.

		Per 100 grm. fresh weight.			Magnesium % dry weight.
		Dry weight.	Magnesium.		
		gram.	gram.	mg. equivalents.	
Dec. 17, 1930.					
Soil	{ Leaves	3.21	0.0123	0.51	0.38
	{ Young joints	3.52	0.0180	0.75	0.51
	{ Old joints	4.98	0.021	0.87	0.42
Sand culture	{ Leaves	3.57	0.0070	0.29	0.20
	{ Young joints	3.44	0.0102	0.42	0.30
	{ Old joints	5.38	0.0232	0.97	0.43
Dec. 19, 1930.					
Soil	{ Cortex	5.51	0.0081	0.337	0.15
	{ Bundle zone	3.28	0.0091	0.38	0.28
	{ Pith	2.00	0.0084	0.35	0.42

*Aluminium.**Quantitative.*

TABLE IV.

		Per 100 grm. fresh weight.			Aluminium % dry weight.
		Dry weight gram.	Aluminium.		
			gram.	mg. equivalents.	

<i>Kleinia articulata.</i>							
Feb. 19, 1931.							
Soil for six months, pre- viously sand culture.	{	Leaves	3.05	0.0054	0.60	0.18	
		Stems:					
		Sample A	3.58	0.0029	0.32	0.081	
		Sample B	3.57	0.0031	0.34	0.084	
Feb. 21, 1931.							
Soil	{	Leaves	3.05	0.0037	0.41	0.12	
		Young joints	3.71	0.0017	0.19	0.046	
		Old joints	4.47	0.0027	0.30	0.06	
Feb. 24, 1931.							
Miscellan- eous, mostly in soil.	{	Cortex	7.68	0.00425	0.47	0.055	
		Bundle zone	3.78	0.00114	0.13	0.039	
		Pith	2.45	0.00088	0.10	0.037	
<i>Rochea versicolor.</i>							
		Leaves	10.65	0.0083	0.92	0.078	

Leaves contain nearly twice as much aluminium as stems, the young joints of February 21 rather less than the old joints. The highest concentration of aluminium is found in the cortex, where it is four or five times as great as in the other tissue samples. Since the concentration in the cortex is of the same order as the concentration in the leaves it is probable that aluminium is associated with the green assimilating tissue.



In view of Hempel's discovery that *Rochea falcata* contains more aluminium than any of the other succulents examined by her, the aluminium content of leaves of a *Rochea* were determined by the new, colorimetric method. The results given at the end of Table IV indicate a much higher concentration than in *Kleinia* (i.e. assuming the aluminium to be in solution in the sap, e.g. as aluminium malate. There is, at least, a larger amount of aluminium reckoned on a fresh weight basis, but the percentage dry weight is higher than in *Kleinia*, so that the percentage of aluminium in the dry substance is not outstanding).

#### Iron.

The amount of iron was too small to estimate quantitatively. Qualitative data obtained during aluminium estimations indicated that the leaf contains much more iron than the stem, and the cortex more than the other tissues of the stem, the pith only the merest trace. Iron, like aluminium, thus appears to be associated with the green tissues.

#### Ammonia.

Molisch's microchemical method gave negative results, so that the ammonia content of the tissues of *Kleinia articulata* must be small.

#### Quantitative.

TABLE V.

		Per 100 grm. fresh weight.			
		Dry weight. grm.	Ammonia—N.		Ammonia—N. % dry weight.
			grm.	mg. equivalents.	
Nov. 13, 1930, 9.30 a.m.					
Soil	{ Leaves	3.89	0.0004	0.07	0.0103
	{ Stem	4.30	0.0004	0.07	0.0093
Nov. 20, 1930, 9.30 a.m.					
Sand culture with extra $\text{Ca}(\text{NO}_3)_2$	{ Young joints	4.93	0.0011	0.20	0.022
	{ Old joints	6.65	0.0019	0.34	0.029

These data confirm the inference that the ammonium content is small, so that this base cannot be of any appreciable importance in causing fluctuations in titratable acidity.

#### Nitrate.

(a) *Microchemical*: application of the diphenylamine reaction to sections and excised portions of tissue gave the following results:

Epidermis: none.

Outermost cortex: very faint colour.

Middle cortex: very faint colour.

Innermost cortex : slightly stronger reaction.

Bundle zone : strong reaction.

Outer pith : very strong reaction.

Central pith : very strong reaction.

The same distribution was found near the apex and at the base of a stem, in young and in old joints. There were, however, indications of an increase in concentration from the apex to the base. The leaf (both lamina and petiole) showed much less than the stem. The inflorescence axis was moderately rich and showed a distribution similar to that in the vegetative axis.

(b) Quantitative.

TABLE VI.

		Per 100 gramm. fresh weight.			
		Dry weight.	Nitrate-nitrogen.		Nitrate-N.
		gramm.	gramm.	mg. equivalents.	% dry weight.
Nov. 20, 1930, 9.30 a.m.					
Same material as for ammonium determinations of same date	Young joints	4.93	(a) 0.023	5.14	0.466
			(b) 0.024		
	Old joints	6.65	(a) 0.044	9.64	0.662
			(b) 0.046		
(a) Colorimetric method.					
(b) Aspiration method.					

Nov. 18, 1930, 9.30 a.m.

		Nitrate-nitrogen per 100 gramm. fresh weight.	
		gramm.	mg. equivalents.
Sand culture with extra $\text{Ca}(\text{NO}_3)_2$	Young leaves	0.0149	3.2
	Older leaves	0.0134	2.9
	Stem: apical piece	0.053	11.3
	middle piece	0.074	15.9
	basal piece	0.087	18.6

Nov. 19, 1930, 9.30 a.m.

Sand culture with extra $\text{Ca}(\text{NO}_3)_2$	Cortex	0.044	8.6
	Bundle zone	0.089	19.1
	Pith	0.072	15.4

These results confirm the increase in nitrate content from tip to base and the lower concentration in the leaves. The results for separated zones show that the cortex contains much less than the pith and bundle zone. The material available for the tests had been grown with additional nitrate and was probably as a whole richer in nitrate than the material used for the microchemical tests.

*Sulphate.*

When the ash of *Klewinia* is dissolved in hot HCl there is a marked smell of  $\text{H}_2\text{S}$  which must come from sulphides formed by the reducing

action of the carbon, formed during incineration, on sulphates originally present.

Quantitative estimations gave the following results.

TABLE VII.

		Per 100 grm. fresh weight.	
		grm. SO <sub>4</sub> .	mg. equivalents SO <sub>4</sub> .
Nov. 27, 1930, 10 a.m.			
Soil	Cortex	0.1376	2.87
	Bundle zone	0.140	2.91
	Pith	0.042	0.87

The pith is definitely poorer in sulphate than the other tissue zones.

#### Chloride.

Microchemical localization by silver nitrate was interfered with by the presence of malic acid, which was precipitated in abundance all over the section. Since, however, the precipitate became slightly purplish on exposure to light, and silver malate itself does not, there must have been a little chloride along with the malate. No useful indications of localization were obtained.

#### Quantitative.

TABLE VIII.

		Per 100 grm. fresh weight.		
		Dry weight.	Chloride.	Grm. Cl. %
		grm.	grm. Cl.	dry weight.
			mg. equivalents.	
Dec. 12, 1930, 9.30 a.m.				
Soil	Leaves	2.85	0.217	6.11
	Cortex	6.46	0.111	3.12
	Bundle zone	3.16	0.170	4.78
	Pith	1.90	0.125	3.52

#### Oxalic Acid or Soluble Oxalate.

The amount of oxalic acid is very small in *Kleinia articulata*, but a fairly definite indication of its localization was obtained by placing sections in a hot 10 per cent. calcium nitrate solution. The oxalic acid or soluble oxalate, so far as could be ascertained, was confined to the outermost tissues of the stem, especially the hypodermis, and occasionally in a few cells immediately below the hypodermis proper.

This result was confirmed by shaving off the epidermis and hypodermis. There was a definite and well-defined reaction when the outer tissues of young plants were tested. With increasing age the quantity

diminished until in old plants it was very small indeed. This decrease in soluble oxalate is accompanied by increase in size of the calcium oxalate crystals in one or more hypodermal layers of collenchyma.

No oxalic acid or soluble oxalate could be demonstrated in the tissues inside the hypodermis.

### *Inulin.*

The localization of inulin was determined by immersing sections in fairly strong alcohol for about 24 hours. When examined a thick white circular zone was evident to the naked eye. This zone corresponded in position to the bundle zone, and the cells were packed with large sphaerocrystals of inulin, together with smaller granules of the same substance.

When treated with Molisch's reagent ( $\beta$ -naphthol sulphuric acid) the inulin dissolved away, leaving a beautiful violet ring. The same reaction was obtained using a fresh section in which the inulin has not been precipitated. When treated with Green's reagent (alcoholic phloroglucin) followed by gentle warming in strong HCl, a reddish-brown ring was obtained.

The distribution of inulin in the aerial stem is remarkably uniform, being confined to a zone, which may vary slightly in width, composed of interfascicular parenchyma plus a layer or two of the perimedullary cells on the inside, and of the inner cortex on the outside.

Thick rhizomes store a larger amount of inulin: here the inulin zone is much wider than in the aerial stem, occupying relatively more of the inner cortex and outer pith, in addition to the bundle zone.

### DISCUSSION.

From the results detailed in the foregoing account certain broad conclusions may be drawn.

Considering first the stem as a whole, the bases and acids that bulk most largely in the sap are calcium, potassium, sodium, nitrate, and malate. Less in amount are phosphate, sulphate, and chloride, while magnesium, aluminium, ammonium, and iron are only present in low concentration. The range of concentrations in each case is, of course, likely to be considerable, as can be seen from the more numerous calcium data given in the previous paper. It has not been possible to determine all the other ions for so wide a range of material. There are the added difficulties that the material used for some was grown in soil and for others in sand culture with nutrient solutions; and that the quantitative data for potassium, sodium, magnesium, aluminium, and chloride apply to the ash from material dried and incinerated as a whole, while the other data are for extracted sap. Although these considerations rob comparisons of some of their

quantitative value, the following rough estimates may serve as an expression of the order of magnitude of the concentrations present; they are given in milligramme equivalents per 100 grm. fresh weight:

Ca, 12; NO<sub>3</sub>, 10; K, 7; Na, 7; Cl, 3.5;

SO<sub>4</sub>, 2.5; PO<sub>4</sub>, 1.5; others, 1 or less.

Malate (by difference), about 10.

An outstanding feature of this list is the high ratio of calcium to monovalent bases.

Turning to the data concerning the localization of these different constituents, we may say that, with the possible exception of chloride, none are uniformly distributed throughout the tissues of the stem.

In the pith, calcium, nitrate, and malate form by far the largest part of the solutes; sodium, potassium, and chloride occur in lower concentration, while the remainder only occur in traces.

In the outer cortex, on the contrary, potassium and sodium are the most abundant bases, combined with nitrate and probably malate when the acidity is high. In the second rank are chloride, sulphate, and phosphate. No soluble calcium can be detected microchemically, and what is found by macrochemical analysis probably all comes from inner cortex included in the samples.

The bundle-zone samples gave the highest proportion of phosphate and nitrate, with moderate concentrations of potassium and sodium; and probably malate. In the second rank come chloride and sulphate. Here also is localized inulin, often in some abundance.

The information regarding malate, other than calcium malate, is rather indirect and not very definite. Precipitation with silver nitrate gave evidence somewhat confused by the presence of chloride. Microchemical tests showed that nitrate was *not* abundant in the inner cortex. As the bundle-zone samples contained a large amount, nitrate is probably specially abundant in the bundle zone proper. Also it may be inferred that the calcium in the inner cortex is present predominantly as malate. The problem of malate distribution, however, requires further elucidation.

The outstanding phenomenon is the accumulation of calcium as nitrate and malate in the pith and inner cortex; but almost as striking is the complementary character of the distribution of the monovalent metals. Indeed, estimating from the average proportions of the three samples into which the stems were divided (for all the experiments the fresh weights averaged: cortex 43 per cent., bundle zone 18 per cent., pith 39 per cent. of the whole) the pith contains some two-thirds of the total (soluble) calcium, the cortex about two-thirds of the total potassium.

The high proportion of calcium in the pith is still further emphasized when taken in conjunction with the low dry weight of this tissue. The

averages of the data available for the dry weight of the different zones are : cortex 7.2 per cent., bundle zone 3.7 per cent., pith 2.15 per cent.

The high ratio of calcium to monovalent bases in the stem as a whole has been pointed out. The ratio is still higher in the pith, but is reversed in the cortex. Thus, taking the data as they stand, the ratios are :

$$\frac{\text{Ca}}{\text{K} + \text{Na}} : \text{cortex } 1/4 ; \text{pith } 4/1$$

$$\frac{\text{Ca}}{\text{K}} : \text{cortex } 1/2 ; \text{pith } 10/1.$$

Such a contrast within a single stem is difficult to harmonize with views on the causation of succulence based upon Ca/K ratios. A *low* Ca/K ratio resulted in increased water-content in the experiments of Pearsall and Wray (10) with *Eriophorum*, and in a large increase in the epidermal water tissue of *Tradescantia fluminensis* in Chapman's experiments (4). Succulent fruits have a low Ca/K ratio. J. W. Brown's analysis of apples (2) gave ratios varying from 0.028 to 0.057. Ratios calculated from Benoy's data (1) for the edible part of some other fruits are : for dates, 0.143 ; for raisins, 0.054 ; for jujubes, about 0.1. In *Kleinia*, on the other hand, it is just the water tissue most characteristic of succulent organs that exhibits the *high* Ca/K ratio—exceptionally high. Moreover this ratio increases with age, through increase in calcium and displacement of potassium (cf. Fig. 1, p. 7). Explanations in terms of increased water-retaining capacity of colloidal cell constituents or their deflocculation in presence of Na or K ions, or the solubility of sodium and potassium soaps, cannot apply in the present case, nor to other succulents containing a high concentration of calcium. For *Kleinia articulata* is not alone in having a high calcium content and a high proportion of soluble calcium to monovalent bases. Hempel found for the succulent leaves of *Cotyledon linguaefolia* 11.6 to 13.8 mg. equivalents of calcium per 100 grm. of sap, only 0.92 to 1.71 mg. equivalents of sodium and potassium together, and a ratio Ca/K + Na varying from 7 to 15. High concentrations of calcium were also found by her for *Cotyledon obvallata* (9.38 to 11.14 mg. equivalents) and *Rochea falcata* (7.28 to 8.96). In *Mesembryanthemum* different cells of the water tissue must have widely different ratios, some containing abundant calcium in solution, others soluble oxalate (13).

The accumulation of nitrate places *Kleinia articulata* among nitrate plants.

The more or less uniform abundance of the nitrate, except for a diminishing concentration in the cortex, suggests a diffusible ion, utilized in the assimilating tissue ; but the concentration is probably less also towards the centre of the pith (cf. Fig. 2).

The general character of the distribution of these outstanding ions is

represented graphically in Fig. 2. The diagrams are not intended as quantitative curves, nor are they mutually comparable as to scale, but they

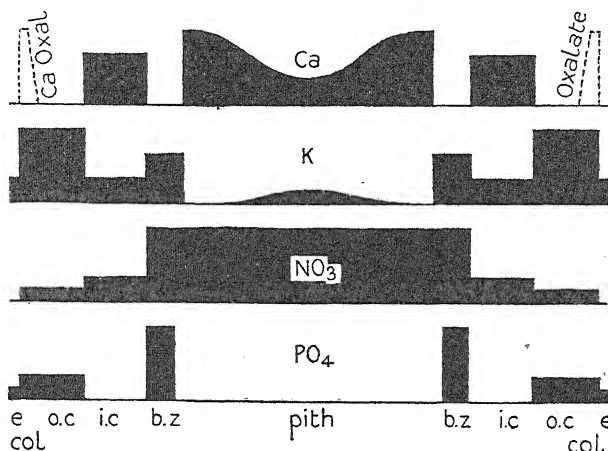


FIG. 2. Rough diagrammatic representation of relative concentration in different tissues respectively of Ca, K,  $\text{NO}_3$ ,  $\text{PO}_4$ , as indicated by microchemical tests: *e.*, epidermis; *col.*, collenchyma; *o.c.*, *i.c.*, outer and inner cortex; *b.z.*, bundle zone.

give a general impression of the relative abundance in different tissues, as far as can be estimated by the eye in a microscopical preparation.

The results of quantitative analyses of separated zones are presented graphically in Fig. 3. As between the different curves the remarks at the beginning of this discussion apply: they are not exactly comparable, as the analyses were made on different lots of material at different times. The broad features, however, are reliable.

A point of some theoretical interest is that the monovalent bases K and Na are similarly distributed; so also are the trivalent bases Al and Fe. Any such relation between the divalent Ca and Mg is obscured by the inclusion in the quantitative analyses of combined Mg which raises its percentage in the green cortex. Both are present in soluble form in greater concentration in the pith than elsewhere, but within the pith they show a complementary relation.

The principal data are collected together in Table IX. Totalling the figures for bases and acids respectively, both cortex and pith show a considerable excess of bases, which must be balanced in the plant by organic acid, chiefly malic.

The fact that the bundle-zone figures show an excess of anions probably means that the anion determinations have been weighted by chance in the selection of material. It is very probable that the nitrate figures are too high, as the material used (all that was available at the time) had received additional nitrate. This would mean that a larger excess of bases and of

compensating malate should be assumed as corresponding to the other data.

Such figures, of course, for the reasons already given, have little absolute value. They do, however, show clearly how great the contrasts may be between different zones, and indicate the general character and direction of the differences.

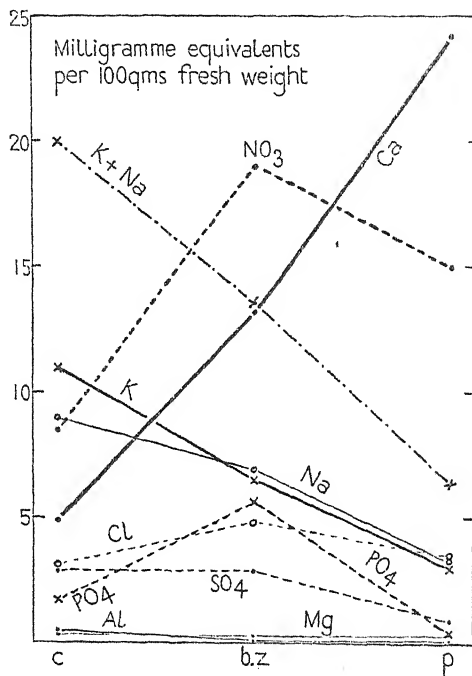


FIG. 3. Graphic summary of quantitative data for separated zones: c., cortical zone; b.z., bundle zone; p., pith.

In view of the elaborate researches of recent years on the mechanism of ionic interchange and of the accumulation of ions inside a cell in higher concentration than outside, which have made abundantly clear the difficulty and complexity of the problem, it would be out of place for us to attempt a lengthy discussion of this aspect of the phenomena we have described. More experimental work than we have so far been able to attempt would first be necessary, to reveal the conditions governing the accumulation and retention of the different ions. Suffice it to emphasize again the great contrasts that exist side by side in the same organ. It is especially interesting that, according to Steward (12), accumulation is dependent upon respiration, and requires expenditure of energy. We may, perhaps, be well advised, for the present, to regard differences like those revealed in the *Kleinia* stem as signs of a differentiation of physiological activity rather



than as results of the action of simple physico-chemical forces in passive osmotic systems, even if in the ultimate analysis the distinction might disappear.

TABLE IX.

Material.		Milligramme equivalents per 100 grm. fresh weight.		
		Cortex.	Bundle zone.	Pith.
Calcium	. . . In soil: sap (13, Table VI)	4.9	13.2	24.2
Potassium	. . . In normal sand: ash.			
	Young:	10.3	5.8	3.5
	Old:	11.6	7.2	2.2
Sodium	. . . In soil: ash	9.0	6.9	3.3
Magnesium	. . . In soil: ash	0.34	0.3	0.35
Aluminium	. . . Mostly in soil: ash	0.51	0.13	0.10
Phosphate	. . . In soil: sap (13, Table VI)	1.72	5.62	0.40
Nitrate.	. . . In sand with extra Ca nitrate: sap	8.5	19.0	15.0
Sulphate	. . . In soil: sap	2.87	2.9	0.87
Chloride	. . . In soil: ash	3.12	4.8	3.5
Titrateable acid (corrected for phosphate)	. . . In soil: sap 3.30 p.m. (13, Table VI)	2.1	2.7	0.5
Total bases	. . . . .	25.7	27.0	30.7
Total mineral acids	. . . . .	16.2	32.3	19.8
Excess of bases	. . . . .	+9.5	-5.3	+10.9

## SUMMARY.

Following up the previous investigation (13) of the distribution of calcium and phosphate in the stem of *Kleinhia articulata*, other solutes have been studied. As far as possible both analytical and microchemical methods were employed. Data were obtained for the following: Inulin,  $\text{NO}_3$ , K, Na, Cl,  $\text{SO}_4$ ,  $\text{NH}_4$ .

In the stem as a whole, calcium, potassium, sodium, nitrate, and malate are the most important ions.

The calcium present is nearly equivalent to the monovalent bases, so that the ratio of calcium to the latter is high.

Analyses of separated zones gave as the  $\text{Ca/Na} + \text{K}$  ratios (in terms of equivalents): for the pith, about 4/1; for the cortex, about 1/4. As no calcium can be detected microchemically in the outer cortex the ratio there must be actually much lower.

The cortex contains about two-thirds of the total potassium, the pith about two-thirds of the total calcium.

Nitrate was found generally distributed, diminishing in abundance outwards in the cortex, and probably less abundant also in the middle of the pith.

Inulin, like phosphate, is localized in the bundle zone. Sugars occur only in very low concentration.

Chloride and sulphate occur in moderate concentration, the former in all three zones in similar proportions, the latter in definitely lower concentration in the pith.

Magnesium, aluminium, iron, and ammonium occur in small concentration, the last two only in traces. Aluminium and iron are associated with the green tissues. Soluble magnesium has been found, particularly in the centre of the pith where there is less calcium.

An excess of bases, alike in cortex and in pith, indicates that a large amount of malate is present in both. The diurnal changes in acidity occur predominantly in those parts of the stem where the malic acid is associated with potassium.

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# **Ecballocystopsis indica n. gen. et sp., a New Member of Chlorodendrales.<sup>1</sup>**

BY

M. O. P. IYENGAR.

With Figures A to R in the Text.

THE alga forming the subject of this communication<sup>2</sup> was growing together with *Ecballocystis courtallensis*,<sup>3</sup> on a wet rock sprayed by the Honey Falls at Courtallum in South India. Although very closely related to *Ecballocystis*, it differs from this genus in possessing a filamentous habit, instead of the dendroid one characteristic of that genus (cf. Fig. L). This difference of habit results from differences in the method of division and behaviour of the daughter-cells, as will be apparent from the subsequent description.

The oblong-elliptic cells are attached end to end to form a long filament which often exhibits characteristic loops in the middle (Fig. L). The mature cells which are 11-16 $\mu$  broad and about one and a half to three times as long, closely resemble those of *E. courtallensis*. Young cells contain from four to eight elongate parietal chloroplasts (Figs. K, R), while in the fully developed ones the number increases to sixteen or thirty-two (Figs. I, G). The chloroplasts in the mature cells are disc- or lens-shaped with a very minute inconspicuous pyrenoid embedded in each (Fig. G). A single nucleus is suspended in the centre of the cell by means of numerous radiating cytoplasmic threads (Fig. F) which appear to be attached to the several chloroplasts. At the two poles the membrane shows small nodular thickenings (Fig. P) which are more obvious in the older cells (those of the second generation and onwards) and more prominent at the lower than at the upper pole. The rest of the membrane is relatively thin. The filament is attached to the substratum by a thick mucilaginous pad secreted by the lowermost cell (Fig. Q). This pad is very similar in appearance to that found in the same position in *E. courtallensis*.

<sup>1</sup> From the Department of Botany, East London College, University of London.

<sup>2</sup> I am indebted to Mr. R. V. Narayanaswamy for material of this alga.

<sup>3</sup> Iyengar, Ann. Bot., xlv, 1932, 204-7.

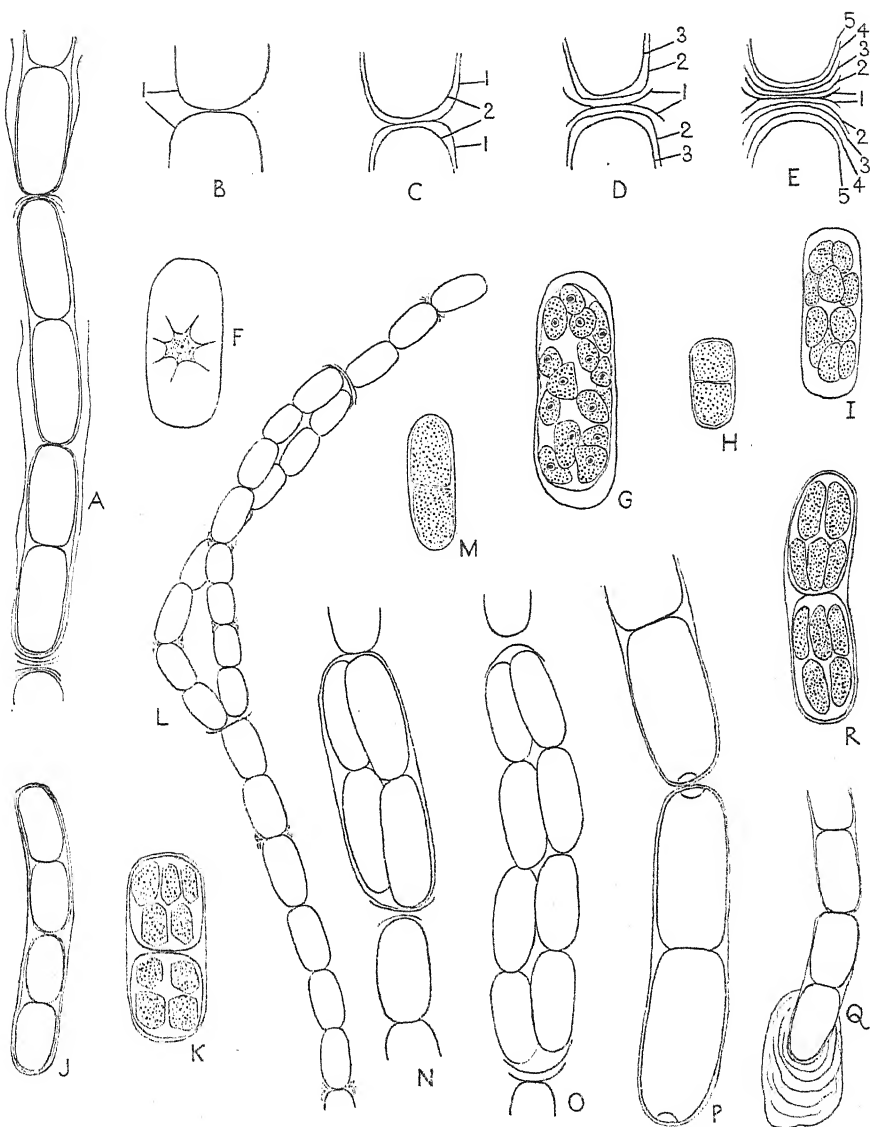
*Cell-division and method of growth of the colony.* The division of the protoplast is very nearly transverse, though a slight indication of obliquity can be detected (Figs. M, H). The two daughter-protoplasts soon surround themselves with separate walls of their own, inside the parent.

In *Ecballocystis* the oblique division of the protoplast and the attachment of the daughter-cells to the wall of the parent-cell by a basal secretion of fairly tough mucilage results in the lower daughter-cell growing past the upper one. Since this process is repeated in successive generations a dendroid colony is formed.<sup>1</sup> In the present alga the daughter-cells do not attach themselves to the wall of the parent-cell in this way. In fact, as explained below, the wall of the parent-cell undergoes complete gelatinization except at its two ends, and very soon disappears. As a result of the practically transverse division of the protoplast the elongating daughter-cells remain the one below the other. A small quantity of mucilage seems to be secreted at the base of each daughter-cell, but this merely helps to keep the cells attached to one another end to end. The wall of the parent-cell becomes much distended, but does not rupture immediately. The new cells grow, and in their turn form pairs of daughter-cells. Occasionally the old membrane of the cell of the first generation may be seen quite intact, enclosing the four cells of the third generation, pairs of which are enveloped by walls of the second generation (Fig. J). Usually, however, the wall of the first generation becomes ruptured when the cells of the second generation are formed, the rupture taking place, not apically as in *Ecballocystis*, but a little below the apex (Fig. A). Soon after most of the side portions of the original wall gelatinize from above downwards and disappear, the only parts of the wall of the first generation to persist being the two concave end-pieces. As the cells of the third generation divide, the two end-pieces of the first membrane become farther removed from one another.

These persisting end-pieces of the walls of successive generations undergo some slight gelatinization, but remain distinctly recognizable for quite a long time as a number of lamellations between adjacent cells. It is possible that they help to hold the cells together. Fig. B shows the end portions of the walls of two adjoining cells. When each of these has formed a pair of daughter-cells, the two pairs of walls will be visible at this point, as in Fig. C, where 1 represents the walls of the original cells, and 2 those of their daughters. When the latter in their turn form cells of a new generation, three pairs of walls will be found as in Fig. D, where 1, 2, and 3 represent the walls of the cells of the first, second, and third generations respectively. In Fig. E portions of the walls of five generations are seen.

Not infrequently the contents of some of the cells divide into four (Fig. N), the daughter-cells appearing as two parallel pairs in which the

<sup>1</sup> Iyengar, loc. cit., 206.



*Echallocystopsis indica* sp. nov. A, portion of filament showing the rupture of the wall of an earlier generation towards the top; B-E, accumulation of end-pieces of walls of successive generations; F, cell with nucleus, chloroplasts omitted; G, cell with chloroplasts and pyrenoids; H, M, division of protoplast; I, K, R, cells with chloroplasts; J, intact wall enclosing four cells belonging to two successive generations; L, filament showing loop-formation; N, division into four; O, beginning of loop-formation as a result of cell-division into four; P, cells showing nodular thickening of wall; Q, base of filament with adhesive gelatinous pad. A, M, J, Q  $\times 450$ ; B-G, I, K, N  $\times 890$ ; H, O  $\times 570$ ; L  $\times 295$ ; P, R  $\times 910$ .

two cells of a pair are attached end to end. When the cells of these pairs in their turn form daughter-cells, two short more or less parallel filaments are produced, as in Fig. O. Further divisions in these filaments lead to the formation of a larger or smaller loop (Fig. L).

As in the case of the species of *Ecballocystis*, no indications of the formation of motile spores have been found. Nor were any sporangial stages observed in the material. The method of reproduction of the alga thus remains to be established.

The absence of the dendroid habit, typical for *Ecballocystis*, is combined with the practical disappearance of the oblique division customary in that genus. The alga here described also differs from *Ecballocystis* in the non-disappearance of the apical portions of the walls of the cells of successive generations, although the lateral (longitudinal) walls gradually undergo complete gelatinization. Nor do the daughter-cells become attached to the wall of the parent. Certain other features of *Ecballocystis* are, however, retained, viz., the presence of several chloroplasts with pyrenoids, the polar thickenings of the wall, and a certain degree of polarity. The last feature is indicated by the rupture of the parent-wall near the apex, i.e., towards the upper end of the filament. The differences from *Ecballocystis*, combined with the *filamentous habit*, which is unusual among Chlorodendrales, warrants the establishment of a new genus which may be called *Ecballocystopsis*. The latter is no doubt closely related to *Ecballocystis*, but it appears to have departed appreciably from the ordinary construction of that genus and to have evolved in a new direction.

*Ecballocystopsis* gen. nov.

Colony filamentous; cells similar to those of *Ecballocystis*, dividing into two or four, division of the protoplast almost transverse, and being followed sooner or later by rupture of the parent-wall a little way below the apex; daughter-cells not attached to wall of parent-cell, the lower remaining permanently below the upper one, so that the cells of successive generations are arranged to form a row; walls of successive generations gradually gelatinizing, except at the two ends, the end pieces accumulating and indicating the number of divisions; reproduction unknown. Motile stages probably lacking.

DESCRIPTION.

*Ecballocystopsis indica* sp. nov. (Figs. A–R).

Cells oblong-elliptic, with 4, 8, 16 or 32 chloroplasts and walls showing polar thickenings. Filaments attached to the substratum by a thick mucilage pad secreted by the lowermost cell; certain filaments showing

loop-formation due to occasional division into four. Method of multiplication not known. Cells 11-16  $\mu$  broad and one and a half to three times as long.

*Habitat.* On a rock constantly sprayed by water from the Honey Falls at Courtallum in S. India, growing together with *Ecballocystis courtallensis* (R. V. Narayanaswamy).

In conclusion, the author wishes to express his great indebtedness to Professor F. E. Fritsch, F.R.S., for his guidance and help in preparing this paper.





# Studies on the Formation of Tubers and Other Storage Organs.

## The Influence upon Translocation of the Period of Light and the Supply of Potassium.

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AND

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With Plate I.

### CONTENTS

	PAGE
INTRODUCTION . . . . .	28
I. THE METHOD OF EXPERIMENTATION . . . . .	29
2. THE RESULTS OBTAINED . . . . .	31
Expt. 1. 1929, <i>Phaseolus multiflorus</i> . . . . .	31
(a) Observational Data . . . . .	31
(b) Quantitative Data . . . . .	32
(c) Potassium Content of Tissues . . . . .	33
(d) Starch Formation and Translocation . . . . .	35
Expt. 2. 1930, <i>Dahlia</i> —2 Varieties . . . . .	36
(a) Observational Data . . . . .	37
(b) Quantitative Data . . . . .	37
(c) Tuber Formation and Dry Matter Content . . . . .	38
(d) Potassium Content of Tissues . . . . .	39
Expt. 3. 1931, <i>Stachys tuberosa</i> . . . . .	39
(a) Observational Data . . . . .	40
(b) Quantitative Data . . . . .	41
(c) Potassium Content . . . . .	43
3. GENERAL DISCUSSION OF RESULTS . . . . .	44
(a) The Influence of the Period of Light upon Tuber Formation . . . . .	44
(b) Translocation to Tuber and Root . . . . .	45
(c) Potassium Economy . . . . .	46
4. SUMMARY . . . . .	50
LITERATURE CITED . . . . .	51

## INTRODUCTION.

THE storage of reserve food substances by flowering plants is frequently associated with the development of fruit and seed or tubers produced either on the stem or root. Undoubtedly, many factors directly, or indirectly, influence the storage organ in its formation and during development. One factor is the length of the daily period of light to which the plant is exposed, this may control the production of flowers and the subsequent fruit and seeds; and also, as will be shown conclusively, it may regulate the rate of formation of the vegetative organs concerned with the storage of food.

It is unnecessary to review the literature dealing with the influence of the period of light upon flowering, for several reviews are already available (see 31), but a very brief summary of some published reports dealing with the influence of this factor upon the growth of tubers may not be out of place.

Krasnosselsky-Maximov (13), referring to the results of Rasumov and others, stated that under the climatic conditions of a Northern European summer such Mexican plants as *Solanum demissum*, *S. Bukasovi*, *S. acaule*, *Oxalis tuberosa*, and *Ulucus tuberosus*, although they grew in a generally satisfactory manner, produced no tubers. The reduction of the daily period of light to 10 hours caused tubers to be formed, but when such plants were again subjected to long days the induced storage of food ceased.

Similarly, of the collection of solanum plants made by the late Martin Sutton, many grew quite well in our latitudes, but several failed to develop any tubers.

In earlier reports (31, 32, 33) it has been shown that, with artichokes, shortening the daily period of light to 10 or 12 hours' duration decreased the rate of elongation of the sub-aerial stems, but accelerated the growth of the tubers. Prolongation of the light period by weak artificial light caused an increase in the rate of elongation of the stems, but a decreased rate of development of the tubers. The more rapid accumulation of carbohydrate in the tubers brought about by adjusting the light period was shown by an increased weight and size of tuber, but not by a higher concentration of inulin in the tuber. Associated changes occurred in the chemical composition of the sub-aerial stems. Confirmatory results were obtained with several varieties of dahlia and also with such starch-producing plants as the potato and runner bean, where the storage took place in wood-parenchyma developed in secondary thickened roots.

The U.S.S.R. workers (13) have demonstrated that these reactions can be localized in the plant to one particular part of the underground stem system. But taking the entire plant as a whole, the rate of tuber pro-

duction throughout any given period of time represents the net balance between the carbohydrate manufactured and that used for growth of sub-aerial and underground structures; as an approximation:

Storage in tubers = carbohydrate manufactured—growth requirements—other local requirements<sup>1</sup> + residual carbohydrate of 'seed'.<sup>2</sup>

The complex factor, 'length of day', influences the total amount of carbohydrate manufactured, the period in which only respiration proceeds, and by regulating growth also governs the utilization of such compounds. Therefore the net rate of storage in tubers or other organs is readily controlled by this technique. Problems of translocation are closely linked with the question of rapid storage of food reserves, and in this connexion some preliminary observations have already been made. It has been shown that at a concentration of 4 per cent. of cane sugar a movement of some 20 cm. per hour is involved in the phloem of the stems of *Phaseolus multiflorus* when subjected to short periods of light. Such a rate of flow is considerably less than that required in the potato or yam.

The conclusive results obtained with cotton by Maskell and Mason (23) have substantiated the claim of the phloem to be considered as the route of translocation. The mechanism of translocation is, however, not clear.

Maskell (21), with potato plants, demonstrated that the rate of translocation depended upon the supply and nature of the supply of potassium available. With additional potassium sulphate the rate of translocation from the leaflets was increased. This rate was also shown to vary with the light intensity and the age of the plants. As the length of the period of light influences the rate of tuber formation, a means is available by which the rate of translocation can be partially controlled; it may be that earlier observations upon the age of the plants and translocation depended somewhat upon the seasonal length of day.

The experiments to be described were commenced five years ago in order to study the influence of potassium upon translocation. For this purpose plants subjected to controlled periods of daylight were grown with and without an adequate supply of potash.

## 1. THE METHOD OF EXPERIMENTATION.

### *Light.*

The technique employed has been described already in this journal. The plants were grown in pots placed on trucks which were moved in and out of a specially constructed dark hut so designed as to be thoroughly

<sup>1</sup> e.g. storage in stems and dormant buds, &c.

<sup>2</sup> Remains of cotyledons or old tubers planted.

well ventilated. Large differences in temperature and humidity were avoided. The following series of plants were grown:

A series. 'Controls.' Received the full natural daylight and were not placed in the dark hut.

B series. Exposed as A from 6 a.m. G.M.T., subjected to electric light intensity 2-3 candle power at soil level (5-6 c.p. at 3 ft.) for 5 (or 7) hours. Total period of light 17 (or 19) hours. 'Long.'

C series. 1930, 1931. Period of light 12 hours. 6 a.m. to 6 p.m. 'Short.'

(In 1929 exposed from 7 a.m. to 5 p.m.—10 hours.)

D series. 1930, 1931. Plants exposed from noon to 6 p.m.—6 hours. 'Very short.'

(In 1929 exposed from noon to 5 p.m.—5 hours.)

The intensity of the supplementary light was low, this precluded photosynthetic activity during this period of illumination as 1929 test leaves indicated. For these tests dahlia and *Phaseolus* were used.

#### *Temperature.*

The nightly shelter of series B, C, and D caused an average increase of 1.9° F. in the minimum temperature over that of A during April, May, and June; in July and August the increase was 1.2° F. The electric light used for 5 (or 7) hours caused an increase of 0.7° F. Such small differences cannot account for the observed results; and, moreover, the observations show that plants of series A and B subjected to the greatest difference of temperature were alike, but they differed from plants of C series. The temperature conditions of B and C series were almost equal.

#### *Water: nutrients.*

All plants received a supply of water believed to be adequate. To compensate for rain that plants of A series only received, it was necessary at intervals to give more water to series B, C, and D.

With sand cultures the surface of the sand was protected from rain by thick paraffin-waxed paper tied around the edge of the pots and fastened around the stems of the plants. Very little water trickled down the stems to the sand. All watering was done by removing these covers and readjusting them. The use of these covers prevented more rapid evaporation from the surface of the sand. (Some observations upon differential wilting of *Stachys* plants grown without potassium in sand are reported later.)

Two types of pots were employed: (a) 12-in. size garden-plant pots containing a rich compost of sandy loam with complete fertilizers; (b) large cylindric pots of glazed earthenware (Doulton's manufacture). These were filled with coarse silver sand after repeatedly washing both pots and sand, above well-washed flints and washed selected drainage material. Before

filling, both the sand and drainage material were tested for potassium by repeated (mechanical) shaking with water and by direct analysis made of finely ground samples. No potassium was found. As plants grown without potassium showed a small gain of this element, a further analysis was made after prolonged digestion of the powdered materials with concentrated acids. The amount of potash found was small, 100 grm. of drainage material yielded 0.013 grm. K. Presumably this was not readily available for the plant, as no potash was found in solution after prolonged shaking with water. The small quantity was present possibly as silicates in the earthenware, flints, and glass vessels. Evidence will be presented which indicates that plants not supplied with a ready source of potassium obtained small quantities from such sources. (Further analyses made after growth showed that no potassium had been added by error or mischance, in using the culture solutions wrongly.)

Through the corked aperture at the base of the pots was inserted a glass tube, bent at right angles and provided with a rubber connexion and clip. This facilitated drainage and provided a means of estimating the amount of solution in the pot. Every fourth day, after drainage, air was forced by pressure through the sand from the base. One of two nutrient solutions was added in measured quantity.

Generally the cultures were successful, the only trouble met with was the presence of algal colonies in the glass tubes. Contaminated tubes were immediately replaced by sterile tubes.

The nutrient solutions (grm. per litre) were as follows:

(1) Potassium solution:

$\text{KNO}_3$ , 1 grm.;  $\text{K}_2\text{HPO}_4$ , 0.27;  $\text{KH}_2\text{PO}_4$ , 0.3;  $\text{NaCl}$ , 0.5;  $\text{CaSO}_4$ , 2  $\text{H}_2\text{O}$ , 0.5;  
 $\text{MgSO}_4$ , 7  $\text{H}_2\text{O}$ , 0.5;  $\text{Fe}_2\text{Cl}_6$ , 0.04.  
 pH 6.4.

(2) Sodium solution:

$\text{NaNO}_3$ , 0.35 grm.;  $\text{Ca}(\text{NO}_3)_2$ , 2  $\text{H}_2\text{O}$ , 0.689;  $\text{NaH}_2\text{PO}_4$ ,  $\text{H}_2\text{O}$ , 0.31;  $\text{Na}_2\text{HPO}_4$ ,  
 12  $\text{H}_2\text{O}$ , 0.56;  $\text{NaCl}$ , 0.5;  $\text{MgSO}_4$ , 7  $\text{H}_2\text{O}$ , 0.5;  $\text{Fe}_2\text{Cl}_6$ , 0.04.  
 pH 6.4.

Half the number (i.e. 12) of pots received potassium, the others sodium, in each series, or light treatment.

## 2. THE RESULTS OBTAINED.

*Expt. 1, 1929. Phaseolus multiflorus—Runner Bean.*

(a) *Development of the plants—observational data.*

The seed sown was selected by size and weight. A sample was taken for chemical analysis. Sowing took place on May 14, the subsequent seedlings were thinned so as to leave two in each culture pot.

When eight weeks old the control plants (A) had attained a height of 47 cm. and bore a few flowers. Plants (B) grown with 12 hours' daylight and electric light were taller (65 cm.) and bore many flowers. Those (C) receiving 12 hours' daylight were short-branched, 'bushy', plants with thick dark green leaves, devoid of any flowers. Similarly (A), those subjected to 6 hours' daylight were short-branched plants without flowers, the leaves were thin and pale green.

The casual observer would not have suspected that any differential treatment in regard to the soil nutrients had been carried out. The differences caused by the light treatment were so outstanding that the smaller differences caused by the nutrient conditions were masked. In the series (C) receiving 12 hours' light, however, a small difference could be detected between the plants receiving potassium and those not receiving this element. The latter were not quite so robust and of a slightly duller colour. Generally, the plants growing in the soil appeared to have slightly thicker and stronger stems than those growing in the sand, whether with potassium or without.

Thirteen weeks from the date of sowing, at the end of August, the plants were carefully removed from the sand, washed, examined, and weighed. They were subsequently dried at 97°C. and again weighed. Potassium determinations were made upon the ash obtained by slow regulated burning.

The roots of the control plants (A) were long and fibrous, and no signs of secondary thickening or food storage was observed in either the series with potassium or without this element. They closely resembled those of plants receiving the electric light, (B series), both with potassium and without.

The plants subjected to 12 hours' daylight, (C), had large thickened roots containing starch, as well as fibrous roots. In this respect plants grown without potassium closely resembled those that had received this element. Translocation to the roots had taken place despite the replacement of potassium by sodium. A similar result was obtained with the plants subject to only 6 hours' daylight (D).

*(b) Quantitative data. (1) Dry weights.*

The total dry weights of the plants receiving the full daylight (A) and those receiving 12 hours' daylight plus electric light (B) were approximately equal. Such plants were heavier than those receiving 12 hours' or 6 hours' daylight. The thickened roots of the latter were heavier than the fibrous root system of the former. These deductions were made after a statistical scrutiny of the data. Fisher's<sup>1</sup> method was employed, by means of which

<sup>1</sup> Statistical Methods for Research Workers, R. A. Fisher, London, 1925, Oliver and Boyd.

the total variance is analysed into parts, the criterion of comparison employed was the difference between the natural logarithms of the standard deviations.

(2) *Percentage composition.*

The data were expressed on a percentage basis of the total dry weight. In Table I average figures are shown. Large differences between the different treatments of light are seen in the percentage of root; the difference between the percentage root of plants with and without potassium are small. The percentage of leaf lamina (petiole and midrib were weighed as 'stem') was more nearly constant, so that the ratio root/'leaf' was governed by the period of light, the values of this ratio were A 0.93, B 0.70, C 2.2, and D 2.5. The ratio serves as an approximation of the mass of translocated food in relative terms of the manufacturing organ, the lamina.

TABLE I.

*Showing Percentage Composition of Runner Beans growing under Various Periods of Light.*

Series.	With Potassium.		With Sodium.		Average.	
	% Root.	% Leaf lamina.	% Root.	% Leaf lamina.	% Root.	% Leaf lamina.
Daylight.						
A Full day . . . . .	26.80	30.60	28.90	29.75	27.85	30.17
B (12 hours + 5 electric) . . . . .	18.80	26.30	19.72	28.80	19.25	27.55
C 12 hours . . . . .	45.80	24.51	56.61	22.30	51.30	23.40
D 6 hours . . . . .	53.40	22.89	59.30	22.61	56.35	22.75
Average . . . . .	36.20	26.1	41.10	25.85	38.60	25.96

*An Example of Method of Statistical Analysis—e.g. per cent. Root.*

	Sum of squares.	Deg. freedom.	Mean square.	Stand. dev.	Log. stand. dev.
Total . . . . .	8274	23	—	—	—
Between 'lights' . . . . .	5783	3	1928	43.9	3.7820
Between 'Na & K' . . . . .	144	1	144	12.0	2.4849
Within replications . . . . .	2347	19	123.8	11.1	2.4048

(c) *Potassium content of tissues.*

The perchlorate method was employed for analysis of the seed and the tissues of the subsequent plants. The data are shown in Table II.

Plants not supplied with potassium showed a small gain of this element. Part of this difference may be due to errors in methods, in multiplication of these errors, &c., but such errors are equally likely to be present in the estimation made of plants supplied with potash. We cannot regard the series as grown *with* and *without* potash, but as 'potash available' and 'potash not readily available'. Some potassium must have been

gained from the glazed earthenware pots, the drainage material, and glass vessels used, despite the precautions taken.

TABLE II.

[Seed data, weight of seed = 1.251 grm.; per cent. moisture = 8.24; ash = 3.818; per cent. K in ash = 39.32; K in one seed = 0.0155 grm.]

*Potassium Content of Tissues—Runner Bean.*

Series & cultural solution.	% K <sub>2</sub> O in Ash.	K per plant, grm.	Ratio of K. in equal (weight) plants grown 'with' and 'without' K. $\chi$
A control. K.	28.00	leaf	0.0639
	28.61	stem	0.1607
	15.00	root	0.0342
			0.2588 $\theta$
			5.84 $\chi$
do. Na.	12.60	leaf	0.0325
	3.75	stem	0.0198
	3.21	root	0.0106
			0.0629
B 12 hours' daylight. + 5 hours' electric.	17.81	leaf	0.1595
	3.50	stem	0.0530
	15.42	root	0.0250
			0.2375
			6.35 $\chi$
do. Na.	11.23	leaf	0.0175
	1.14	stem	0.0096
	8.50	root	0.0057
			0.0328
C 12 hours' daylight.	28.49	leaf	0.1280
	37.62	stem	0.0165
	18.09	root	0.0926
			0.2371
			3.53 $\chi$
do. Na.	7.62	leaf	0.0360
	4.505	stem	0.0150
	5.05	root	0.0104
			0.0614
D 6 hours' daylight.	34.415	leaf	0.0789
	31.64	stem	0.0185
	21.76	root	0.0465
			0.1439
			3.29 $\chi$
do. Na.	9.89	leaf	0.0260
	8.00	stem	0.0059
	8.35	root	0.0120
			0.0439

$\chi$  signifies calculated on equal dry weights or corrected for dry weights.

$\theta$  signifies that more than 10 times this amount of potash was presented to the plants.



The ratio of the potassium present in the tissues of the two series was calculated on a basis of equal dry weights, and is shown in Table II, column 4. In the tall flowering plants (light conditions A and B) approximately six times as much potassium was present in the tissues of plants supplied with this element as in the tissues of plants deprived of potassium. In the short bushy plants with thickened roots the ratio was lower, approximately 3.4, the technique employed had resulted in a relatively small rate of dilution. As far as translocation to the roots was concerned, the available evidence indicated that such a dilution of potassium, in the presence of abundant sodium, was without effect; a dilution of six times produced no readily noticeable effect upon the rate of development of climbing flowering plants, or upon the rate at which carbohydrate accumulated in the young cotyledons of the seed, as judged by a comparison of the roots and seeds during growth.

*(d) Starch formation and translocation.*

The length of the daily period of light under which runner beans are grown has previously been shown to influence the anatomical structure of the leaf, the size of the palisade cells, and the rate of formation of starch. By exposing plants devoid of starch to equal illumination it was shown that more starch accumulated in leaves (either attached to, or detached from the plant) of plants grown under shorter periods of light than in leaves of plants grown under long periods of light. Such observed differences were not to be totally explained by the thickness of the leaves, as more starch was present in the palisade and mesophyll cells of the leaves. Further tests have been made using the plants grown with and without potassium.

For these tests leaflets were selected so that as far as possible they were equal in size, age, and position on the plant. Tests were made by iodine, using colour charts for matching. Replications of at least 4 (usually 6) leaflets were made. The plants were kept in the dark room for 36 hours (at 18°C.) before testing. The periods of exposure were of 4½ and 6 hours' duration. Both attached and detached leaves with petioles in tap water were employed. When tested the plants were (a) eleven, (b) twelve weeks old. The control plants were then tall flowering individuals with developing pods.

In all tests, with both detached<sup>1</sup> and attached leaves, plants grown under 10-hour periods of light produced more starch in the leaf than did plants grown under longer periods of natural or natural and artificial light.

Such differences were much larger than those between plants grown

<sup>1</sup> In certain cases, apparently irregularly, the stomata of detached leaves remained closed for a longer period than those of attached leaves, although leaves were detached 2 hours before exposure. Tests were made by porometer and the strip method, and direct microscopic examination.

with and without potassium. With attached leaves exposed, for  $4\frac{1}{2}$  and 6 hours to daylight, plants grown with potassium contained on the whole slightly less starch. With detached leaves the reverse was generally observed. This was best seen in the '12 hour' series C. Further tests made after returning the plants to the dark room did not give significant results. Generally comparisons made within light treatments (K v Na) were barely significant.

All that can be concluded from these tests is that some evidence was obtained that potassium may facilitate starch formation and translocation. The period of light under which the plants were grown undoubtedly affected the organization of the leaf, and its rate of formation of starch. As similar tests were not made with other species the relationship of these observations to those of other investigators may be here discussed briefly.

Hartwell (7) reported starch congestion with an adequate supply of mineral salts, but also suggested that other disturbing factors may be the cause. The scanty evidence collected from these tests was not supported by the observations made upon the rate of storage of starch in the roots where no differences were observed between plants grown with and without potassium.

These observations are in close agreement with those of Lubimenko (16), who observed differences in starch formation in attached and detached leaves due to the period of light under which the plants were grown. Weaver and Himmel (36) found that in sunflower and radish and red clover a larger quantity of carbohydrates accumulated in the leaves of plants exposed for longer periods of light; ragweed under long periods of daily illumination showed no higher rates of accumulation of carbohydrates than under 7 hours' daily exposure, frequently lower rates were observed. In dahlias, the increase observed in carbohydrates during a 7-hour period of light was equal to that of plants exposed for longer periods of illumination. As Weaver and Himmel point out in the interpretation of their results, their estimations represent the balance of carbohydrate manufactured over that used in respiration, translocation, and growth, so that a more rapid utilization of the products of photosynthesis under certain periods of light prevented the estimation of the magnitude of assimilation itself. The development of the root system of the plants was also observed by Weaver and Himmel (35), who found that the root system was generally directly correlated with the 'top' growth.

*Expt. 2, 1930. Dahlias—Varieties: 'Goldperle' and 'Epsom Star'.*

The experiment was repeated with this species. To ensure homogeneity of material, cuttings taken from tubers of these varieties were rooted in a sandy medium relatively poor in potassium. These were sub-

sequently selected by size and fresh weight whilst similar samples were dried and weighed, and ground up for analysis.

The series grown with and without potash were (A) 'Control', full daylight; (B) 10 hours' daylight and 5 hours' electric light; (C) 10 hours' daylight; (D) 5 hours' daylight.

Differential treatments commenced on June 1, 1930.

(a) *Observational data.*

In the series 'with potassium' at the end of July there was considerable difference between the growth of the plants receiving long and short periods of light. Thus:

'Goldperle': height in cm. of controls (A) = 50, of electric (B) series = 50, of 10 hours (C) series = 30, of 5 hours (D) series = 24.

Similar differences were observed with 'Epsom Star'. The short plants did not flower freely.

The differences in habit between plants supplied with, and deprived of, potassium was not large at this date. In series A and B little or no apparent differences were recorded. In series C, 10 hours, particularly with variety 'Epsom Star', plants deprived of potassium bore leaves of a lighter colour. Plants of 'Epsom Star' deprived of potassium (A and B series) showed a tendency for the leaves to droop a little on a few occasions. Wilting did not occur, the leaf blades were turgid.

In August careful displacement of the surface sand in the pots revealed that tuber formation was progressing more rapidly in C and D series than in A or B, where no tubers were visible.

The plants of all series were removed on September 22. Careful washing was carried out, and very little of the root system was lost in the process. The tubers of C series were heavier than the longer and narrower tubers of A or B series. Plants exposed to 5 hours' daylight had formed only a few large tubers. The differences observed in size, shape, and fresh weight between the tubers of plants receiving different periods of light were very much more pronounced than those between the plants grown with and without potassium. The plants were weighed fresh (after rapid separation into leaf, stem, tuber, and root); dried, reweighed; and finally ground up for analysis.

(b) *Quantitative data—partly shown in Table III.*

The following conclusions were arrived at after statistical scrutiny of the data by the method of analysis of variance:

*Dry weight.*

(1) The total dry weights of the plants, particularly of variety

'Epsom Star', grown with potassium was greater than that of plants receiving sodium only.

(2) In both varieties, series B, 10 hours' and 5 hours' electric light, closely resembled series A in their general distribution of the dry weight. Series A, controls, were heavier.

(3) Series B differed in general distribution of dry weight from series C, 10 hours only. This held good with and without potassium.

That is, the additional weak electric light caused no significant increase in total dry weight, yet exercised a governing influence on the utilization of the photosynthetic products by modifying growth. This effect was obtained with and without potassium.

TABLE III.

*Showing the Influence of the Period of Light upon the Organization of Dahlia Plants grown with and without Potassium.*

Data as a basis of dry weight.

Series and Variety.	Average of total dry weights as % of control, with potassium.	Ratio of total dry weights K/Na.	With Potassium.		Without Potassium.	
			Leaf % of total.	Tuber % of total.	Leaf % of total.	Tuber % of total.
A Control full day						
Epsom Star	100	1.51	15.94	43.91	20.40	43.70
Goldperle	—	—	19.80	29.20	12.00	32.82
B 10 hours' daylight with 7 hours' electric						
Epsom Star	63.0	1.24	24.94	11.02	29.75	18.69
Goldperle	—	—	29.51	7.39	32.15	8.45
C 10 hours' daylight						
Epsom Star	41.0	1.64	13.78	69.38	18.50	62.61
Goldperle	—	—	14.70	65.24	12.61	71.64
D 6 hours' daylight						
Epsom Star	7.7	1.22	12.50	64.76	6.21	70.40
Goldperle	—	—	19.20	54.05	5.27	46.00

(c) *Tuber formation and dry matter content :*

(1) The average percentage contributed by the tubers to the total dry weight was :

Control, series A . . .	37 per cent.	Electric, series B . . .	12 per cent.
10 hours, series C . . .	95 per cent.	6 hours, series D . . .	64 per cent.

(2) The mass of tubers was actually greater under shorter periods of light, C, than under long periods, A and B, but the percentage of dry matter in the tubers was not influenced. This held true with and without potassium.

(3) The differences observed in percentage dry matter of leaf, stem, tuber, and root, due to the potassium were without significance.

(4) The difference observed in the percentage of dry matter of leaf, tuber, and root due to light treatments were without significance.

(5) In plants receiving long periods of light more dry matter (fibre) was found in the stems than in plants receiving shorter periods of light. This held true with and without potassium.

(d) *Potassium content of tissues.*

After making test analyses, it was decided, owing to the small mass of ash obtained, to 'pool' the material from the different light treatments, and to analyse all the tissues of the plant together. This enabled estimations to be made in triplicate, but unfortunately it precluded the possibility of ascertaining whether or not any differential uptake of this element under different periods of light had taken place. The data obtained are shown in Table IV for the two varieties.

TABLE IV.

Average fresh weight of cuttings = 3.002 gm. (all between 2.75 and 3.35); per cent. dry matter = 18.24; ash content 13.6 per cent. of dry matter;  $K_2O$  in ash = 35.65 per cent.; K as fresh weight = 0.74 per cent.; K present = 0.022 gm. per cutting.

*Potassium contained in Tissues of Dahlia Varieties.*

Variety.	Treat- ment.	% $K_2O$ in ash.	K present in plant (gm.).	Ratio of K. in equal (weight) plants with and without K.
Epsom Star	K.	18.496	0.496 <sup>1</sup>	4.28
	Na.	7.004	0.097	
Goldperle	K.	8.941	0.146	4.85
	Na.	3.400	0.042	

A gain of 0.0755 gm., 'Epsom Star', and 0.020 gm., 'Goldperle', of potassium was made by plants not supplied with this element. Calculating on a basis of dry weights a dilution of 4.5 times was achieved by withholding potassium. Such a dilution, accompanied by an abundant supply of sodium, did not cause an appreciable effect on the proportion of the total dry matter translocated to the tubers, but such a dilution was accompanied by a significant decrease in the total dry weight, this was more clearly observed with the more rapidly growing variety.

*Expt. 3, 1931.* *Stachys tuberifera* Naudin = (S. Sieboldi *Miq.*)

Four series of plants were grown in this experiment, namely:

(1) Seedlings raised from seed sown in late February, transplanted and grown in large pots in a medium loam.

<sup>1</sup> Ten times this weight of K was presented to each plant.

(2) Plants raised from small weighed pieces of tubers, and grown as (1).

(3) Plants raised as (2), but grown in sand with Na culture solution.

(4) Plants raised as (3), but grown in sand with K culture solution.

These series were subjected to the different light treatments A, B, C, D, from May 1.

A further three series were grown in open soil, namely:

(5) Raised from seed as (1) and grown in light loam.

(6) Raised from tubers as (2) and grown in light loam.

(7) Raised from tubers and grown in a cold, stiff, clay soil.

(a) *Observational data.*

(a) *Leaf colour.*

All plants receiving 5 hours' light (D) bore leaves of a light green colour, whilst the leaves of C series (10 hours) were very much darker than those of A or B series (or 5, 6, or 7).

(b) *Height.*

The different periods of light controlled elongation of the stems as shown in Table V.

TABLE V.

*Growth in Height of Stachys subjected to Different Periods of Light, with Various Nutrient Conditions.*

Origin.	Soil.	A Control. Full daylight.	B Electric. 10 hours' and 5 hours' electric.	C 10 hours' daylight.	D 5 hours' daylight.
Average Height, 10 weeks' treatment, cms.					
(1) Seedlings.	Loam.	7.20 ± 0.09 <sup>1</sup>	7.56 ± 0.08	7.20 ± 0.06	7.00 ± 0.05
(2) Tubers.	Loam.	34.14	43.07	22.59	14.06
(3) Tubers.	Sand Na.	27.45 ± 0.48	36.58 ± 0.57	19.02 ± 0.33	13.07 ± 0.24
(4) Tubers.	Sand K.	28.32 ± 0.49	34.51 ± 0.72	18.41 ± 0.50	11.57 ± 0.31
Average Height, 14 weeks' treatment, cms.					
(1) Seedlings.	Loam.	14.09 ± 0.21	16.13 ± 0.20	10.75 ± 0.19	7.58 ± 0.07
(2) Tubers.	Loam.	42.07	49.49	23.75	17.04
(3) Tubers.	Sand Na.	33.40 ± 0.61	38.20 ± 0.58	21.75 ± 0.47	16.22 ± 0.33
(4) Tubers.	Sand K.	33.26 ± 0.58	37.75 ± 0.60	22.04 ± 0.46	16.56 ± 0.31

The plants receiving the longer periods of light grew tall; those grown in sand were slightly smaller than comparable ones in loam. No significance was to be attached to the differences observed between the series differing in the amounts of potassium received.

<sup>1</sup> Probable error of mean.

(c) *Flowering.*

The light treatments produced marked differences in flowering. Control plants, A, and plants subject to 10 hours' daylight and 5 hours' electric light, B, flowered freely; plants receiving only short periods of light, C and D, produced no flowers. This result held for *all nutrient conditions*. Seedlings did not flower so profusely as plants raised from tubers.

In the open ground the differences due to soil conditions were relatively of little significance, flowering was delayed by some four days on the heavy clay soil as compared with the time of flowering of plants on a sandy loam.

(d) *Occasional wilting.*

Despite the fact that the sand in the pots was kept moist throughout the entire growth period, the absence of potash in the supply of available nutrients influenced the water relationship of the tissues. For example, on July 23, a very hot day, at 5 p.m. *all* the plants (48 in number) grown without potash which had been exposed for 10 hours or more to the sun were wilted, both stems and petioles were limp. The result was clear cut, as *all* the comparable plants supplied with potassium were erect and turgid (Pl. I). After further watering and the nightly shade, recovery was apparently complete.

(e) *Formation of 'runners'.*

Plants subject to 10 hours' daylight (C series), raised from seed and tuber and grown in sand and loam, produced a few prostrate branches devoid of expanded foliage leaves, but bearing very small scale leaves. These surface runners did not swell as did underground tubers, they were absent from series A, B, and D.

(b) *Quantitative data.*

The data collected were analysed statistically, and some of the figures are shown in Table VI. Conclusions drawn are based on significant differences.

From the entire data the following conclusions are drawn:

*Dry weight.*

(1) Plants receiving 10 hours' daylight (C) and 10 hours' daylight and 5 hours' electric light (B) produced less dry weight than the (full daylight) controls (A).

(2) A higher proportion of the total dry weight was found in the tubers of the C series than in A series. The actual dry weights of the tubers were approximately equal in these two series.

(3) The difference between the total dry weights of plants grown under all the periods of light with and without potassium was not significant.

TABLE VI.  
*Stachys tubrifera*—Dry Weights.

Origin.	Soil.	A Full daylight.	B 10 hours' and 5 hours' electric.	C 10 hours' daylight.	D 5 hours' daylight.
Total dry weights in terms of control = 100.					
(1) Seedlings.	Loam.	100 (28.86)	89.0	87.4	11.4
(2) Tubers.	Loam.	100 (26.05)	82.5	85.6	46.0
(3) Tubers.	Sand Na.	100 (36.50)	87.0	86.8	29.5
(4) Tubers.	Sand K.	100 (40.29)	73.0	85.0	20.2
% of total dry weights contributed by the tubers.					
(1) Seedlings.	Loam.	63.0	70.0	77.7	55.0
(2) Tubers.	Loam.	55.5	58.90	70.2	61.2
(3) Tubers.	Sand Na.	61.4	63.5	65.8	52.7
(4) Tubers.	Sand K.	64.3	63.0	76.3	64.8

(4) Under periods of 10 hours' daylight (C) and 5 hours' daylight (D) the percentage of the total dry weight contributed by the tubers was less in the case of plants deprived of potassium than in the case of plants supplied with this element.

(5) An increased proportion of tuber was accompanied by a decreased proportion of sub-aerial stem. The percentage contributed by the leaf blade remained approximately constant under all periods of light.

#### *Moisture content.*

(6) No significant difference was observed in the moisture content of the leaves of plants grown with and without potassium at the dates of analysis.

(7) Between the series subjected to different periods of light the more fibrous stems of control plants A contained less moisture than did the stems of C and D series.

(8) The plants receiving 10 hours' daylight and 5 hours' electric light (B series) resembled the controls (A). The differences observed in the percentage of dry matter in swollen tubers were not significant. In unswollen underground stems more dry matter was found in the stems of C and D series than in A and B series.

These observations, made in late summer, afford no indication of the moisture content at any earlier stage of growth. Throughout the entire period the plants grown with and without potassium received the same amount of water. Infrequently differential wilting was observed, so that the transpiration balance, water lost/gained, was influenced by potassium. The differences in the dry weights were so small between the two series that it is concluded that this infrequent wilting was not associated with a serious decrease in photosynthetic activity in this species.



Generally, the analyses of the seedlings confirmed the results from plants grown from tubers; as in (6), but there was no significant difference between the moisture content of the stems as in (7). In the tubers the percentage of dry matter was less in plants subjected to long days than in plants subjected to short days.

The rate of elongation of the stems above ground was greater in the former. Presumably carbohydrate was utilized more rapidly in the former plants (A and B), and stored more rapidly in the short day plants (C and D).

(c) *Potassium content.*

The data obtained by the perchlorate method are summarized in Table VII, which shows the percentage of potassium in the tissues of plants supplied with this element and the ratio of potassium present in the plants grown with and without potassium.

TABLE VII.

Tubers planted. Av. wt. = 3.062 grm.  $K_2O$  in ash = 37.17 per cent.; per cent. ash = 16.6; mass of K in tuber = 0.0067 grm.

*The Potassium Content of the Tissues of Stachys tuberosa, grown under Various Periods of Light.*

Series.	% K in tissues grown with potassium.	Ratio of K in tissues in K/Na series.	Mass of K taken up per plant grm. with K.	Ratio of mass taken up K/Na series.
A Control	2.12	leaf 2.64	0.790	2.65
	2.07	stem 3.65		
	1.74	tuber 1.84		
	3.48	root 6.53		
B 10 hours' daylight 5 hours' electric	3.61	leaf 5.95	0.794	2.50
	3.91	stem 2.50		
	2.17	tuber 2.00		
	2.96	root 2.51		
C 10 hours' daylight	4.39	leaf 6.45	0.911	2.81
	5.06	stem 8.70		
	2.00	tuber 2.00		
	2.94	root 3.20		
D 5 hours' daylight	4.68	leaf 4.51	0.317	2.30
	5.06	stem 3.41		
	2.81	tuber 1.85		
	3.30	root 5.80		

The following conclusions are drawn from the data :

(1) The potassium content of the leaves of plants grown in sand to which potassium was added was greater under shorter periods of light. This was not observed in plants deprived of adequate potassium.

(2) The stems of plants supplied with potassium contained a higher percentage of this element under shorter periods of light, the total mass present was, however, less in the short stems of plants of C and D series.

A progressive increase in the potassium content was not observed with shorter days in plants deprived of an adequate supply of this element. On the contrary, under the 10-hour periods (C series), where rapid translocation to the tubers took place, the percentage of potassium in the stems was very small. The ratio of the potassium present in the two series grown with potassium and with sodium only was high = 8.7.

(3) In tubers the percentage did not vary widely. There was approximately twice as much potassium present in the tubers of plants supplied with this element as in plants deprived of an adequate supply.

(4) In the roots the percentage of potassium did not vary widely in plants supplied with this element.

(5) When the total mass of potassium taken up by the plants supplied with this element is considered, a greater mass was taken up by the shorter and more bushy plants of C series. In these plants translocation to the tubers was rapid. Although the tissues of the small plants grown under 5 hours' of light (D) were relatively rich in potassium, the total weight acquired by the plant was smaller than in the other series.

(6) The dilution of the potassium in the tissues as a whole, brought about by depriving plants of potassium in solution, was only some 2.5 times.

### 3. GENERAL DISCUSSION OF RESULTS.

To obtain a complete record of the process of tuber formation a much larger number of plants would be required for sampling at intervals. The date of sampling was a selected one, the plants were removed when the first signs of autumnal discoloration occurred in the leaves of the control plants. The data collected therefore reveal the progress made by the plants at a late stage in the process of tuber formation—as such control plants had almost ceased from photosynthetic activity. Whilst plants of a winter hardy species subjected to other periods of light might attain this stage at a later date, in the case of dahlias and *Phaseolus multiflorus*, this could not take place.

#### (a) *The influence of the period of light upon tuber formation.*

The three experiments showed the influence of the period of light upon the utilization of the carbohydrate in the plant. The differences obtained with *Phaseolus* and dahlia were more pronounced than those with *Stachys*. Generally, a more rapid rate of growth was accompanied by a decreased rate of storage. With long periods of light of both natural daylight only and daylight supplemented by weak electric light the upward growth was vigorous, and frequently the stems supported floral branches. The rate of tuber formation took place at a normal velocity under long

periods of natural illumination of photosynthetic significance, a subnormal rate of accumulation of food products was observed with long periods of light composed partly of weak light of no photosynthetic significance. It appeared that the long periods of light operated upon the mechanism governing the process of stem elongation. There was little difference generally between the total dry weights of control plants and plants receiving 10 or 12 hours' daylight supplemented by weak electric light. In habit these two series were similar. In plants subject to 10 or 12 hours the rate of apogeotropic growth was checked, and the relative mass of carbohydrates translocated to subterranean stems or roots was greater. In *P. multiflorus* starch congestion of leaves and shoots occurred under short days.

Maximov and Lebedincev (19), working with *P. vulgaris* and *Impatiens* sp., found that the length of the daily period of light influenced the magnitude of root development, short periods accelerated the rate of storage of food; with long periods of light, a more fibrous root system was produced. Cuttings made late in the year stored food substances at the nodes and elsewhere.

Lemmermann and Hitchcock (37) observed the influence of the period of light upon tuber formation in several varieties of dahlias. Weaver and Himmel (36) concluded from their studies that the development of the root system was in direct correlation with that of the subaerial growth, but nevertheless the proportional distribution of the food reserves was controlled by the period of light, and with dahlias short periods of light caused rapid accumulation of food in the tubers; in red clover, iris, radish, and other plants the shorter periods of light retarded the development of shoot and root.

All the available evidence tends to show that this factor, as Garner and Allard (5 and 6) pointed out, governs the utilization of the carbohydrates as well as the magnitude of the photosynthetic activity.

A practical application of such considerations is illustrated by observations made by Cayeux (2), who attempted to propagate certain 'sports' of dahlias by rooting cuttings taken late in the season. Such cuttings immediately formed small tubers.

(b) *Translocation to tuber and root.*

The data obtained in different seasons and from different species were 'pooled' and a comparison made on total dry weights of the underground organs thus:

dry weight control 'A' series = 100; dry weight 'B' series = 60; dry weight  
'C' series = 130; dry weight 'D' series = 22.

When, regardless of season, species, and light treatments, the actual dry weights of translocated material recovered from plants grown with and without potash were compared, the difference obtained was of no (statistical)

significance, i.e. K 100—Na 96.4, but taking each light treatment separately for three species in three years we have :

Series.	With potassium.	Without potassium (Na.).
A Control . . . .	100	100.9
B 10 hours & 5 hours	100	103.4
C 10 hours . . . .	100	83.4
D 5 hours . . . .	100	99.2

Only under the treatment (C), causing the most rapid translocation of carbohydrates to roots and stems underground, did less dry matter accumulate in plants grown without potassium than in plants grown with this element. In the other cases (A, B, D) the differences were without significance. These figures support the earlier conclusions, for each species separately, drawn from a consideration of the proportional distribution of the dry matter.

A similar consideration of the dry weights of the leaves indicated that no significant difference was caused by withholding potassium, so that the differences observed previously in the amount of food substances translocated are not explained by differences in the *leaf mass*. James (9) found that potassium sulphate was without effect upon the *leaf area* of potato plants.

In order to obtain further information as to the efficiency of the leaves the total dry weights of the plants were considered.

It must be pointed out that no respiration data are available.

The total dry weights of all the plants receiving potassium was greater than that of plants grown without this element, the ratio being 100/84. The most susceptible tested plant was the dahlia 'Epsom Star'. Such differential response was noted by Hartwell (8), who gives a list of plants classified by their response to potassium. The greatest difference was observed under the longest period of natural light.

Gregory and Richards (4) with barley recorded a greater respiratory activity in plants deprived of potassium and a decreased rate of assimilation under light of high and low intensity. Although our data cannot be analysed further, it may well be that the difference in the dry weights shown by the control plants grown with and without potassium was primarily due to the influence of the potassium on photosynthesis. A smaller difference was observed when the period of assimilation was shortened to 10 hours.

### (c) *Potassium economy.*

The results obtained from runner beans, dahlia, and *Stachys* are in agreement, in general, with the observations of Lupke (18), Nobbe (26), Reed (28), Russell (29), and others, in that they show that the production of carbohydrates, measured by dry weight of the plant, may be impaired

by a deficiency of potassium, despite an adequate supply of sodium. A decrease of assimilatory activity was demonstrated by Briggs (1) in the absence of certain mineral salts.

The influence of potassium was also seen on the moisture relationship of the leaves of *Stachys*, plants deprived of potassium wilted more readily. Yet no differences were observed in the moisture content of the tissues as a result of potassium starvation. James (9), working with potato, recorded that potassium sulphate was without effect upon the moisture content, he, however, noted that potassium chloride caused an increased water content in the leaves.

In distribution of this element plants supplied with potassium showed a higher concentration per unit dry weight in leaves and stem than in tuber or storage root. As the mass of the tubers increased, a steady accumulation of potassium took place in these storage organs.

By growing plants without an adequate supply of potassium a dilution of three to nine times was brought about. In such plants where translocation to the roots or tubers was rapid a marked decreased concentration of this element resulted in the stems (see *Phaseolus*, 5 hours, and *Stachys*, 10 hours, without potassium). In such cases the movement of potassium would seem to take place against the gradient. James (10) states that the movement of potassium from one organ to another may be either with or against the average concentration gradient, but 'that movement contrary to such gradients is always in the normal direction of the transpiration stream, whilst those with the gradient are against the stream'.

In the plants of *Stachys* supplied with potassium this appears to hold true, but when plants deprived of potassium are considered, it appears that this quoted statement may require modification.

The chemical changes concerned with translocation include hydrolysis and condensation. Loew (15) and Stocklasa (30) suggested that potassium plays the rôle of a catalyst facilitating condensation, and that the radio activity of the element is directly concerned with its influence upon the rate of photosynthesis. James (10) also favours a somewhat similar interpretation, he also suggests the possibility of a continued circulation of part of the potassium.

It would appear from such considerations unlikely that the mass of material translocated should vary as the mass of potassium present in the plant. The data collected in the experiments were examined from this point of view and are shown in Table VIII.

The data show that both with starch plants and inulin plants a wide variation occurred in the ratio, translocated dry matter/potassium. It is therefore somewhat unlikely that in translocation a mass relationship exists between potassium and carbohydrates. Such evidence favours the view that potassium functions as a catalyst and/or that it circulates in the

plant. The other possibility is that as far as translocation is concerned sodium functions equally well; against such an explanation must be considered the data from isolated leaves, the data of other investigators, and particularly the fact that a lack of potassium seemed to prevent rapid translocation under certain conditions.

TABLE VIII.

*Showing the Dry Weight of Roots and Tubers per unit grammes of Potassium in the Entire Plant.*

Plant.	Light treatment.	With potassium.	Without potassium.
<i>Phaseolus multiflorus</i>	Control A series	5.8	80.1
	B "	8.0	61.2
	C "	19.0	61.2
	D "	18.5	100.2
<i>Dahlia</i> , Epsom Star	(A, B, C, D)	22.0	69.0
Goldperle	(A, B, C, D)	8.9	68.5
<i>Stachys tuberifera</i>	Control A series	36.5	78.5
	B "	24.9	71.5
	C "	29.3	75.5
	D "	13.8	40.3

Janssen and Bartholomew (11) observed the ratio of carbohydrates to potassium present in oats and cowpeas, this ratio fell with an increase of potassium; with soybeans the carbohydrate content tended to remain steady, despite decreased potassium. They also considered that the plant may, under certain conditions, take up more potassium than is required.

Nightingale (25) and his associates, working with tomato, reported that a deficiency of potassium caused an accumulation of carbohydrates, this appeared to be associated with a failure to synthesize organic nitrogenous compounds; high concentrations of nitrites were found in the tissues. With beet and other root crops an accumulation of carbohydrates resulted from decreased potassium, but when a decided deficiency of this element existed the rate of manufacture of the carbohydrates also decreased. Janssen and Bartholomew (12) also report a high content of soluble nitrogen in tomato plants deprived of an adequate supply of potassium. The indications are therefore that potassium is also associated with protein metabolism.

Penston (27), by means of microchemical tests, found that there was in the potato a close association between potassium and protein, and observed also an increased potassium precipitate, beyond the normal found in stems, in the swelling tips of stems producing tubers.

The influence upon carbohydrate and protein synthesis is reflected in the relationship between potassium and growth. James (10) found that the potassium present, as a percentage of dry weight, was correlated with the

relative growth rate during the earlier phases of development of the potato plant. At a later stage the rate of growth declined more rapidly than did the potassium content, but the rate of uptake of this element diminished even more rapidly. James suggested that this indicated an accumulation of the potassium in non-meristematic regions of the plant.

The inter-relationship of potassium and light intensity has been studied by Lemmermann and Leisegang (14) in shading experiments. An abundant supply of potassium, more than any other element tested, increased the ability of the plant to utilize diminished light. The effect of additional potash fertilizers reached their maximum under full natural daylight.

Clements (3) working with the 'triangular' system of water cultures made analyses of the plants grown under varying conditions of light and nutrient supplies. He found that exposure to short periods of illumination moved the optimum position in the 'triangle', larger food reserves accumulated in plants grown under short days; and generally there was no fixed 'best' response of plants to nutrients independent of photosynthetic considerations. Trelease and Livingstone (34) consider that the physiological value of salt solutions cannot be stated without reference to climatic factors. Nemec (24) working with oats and rye grown in soil stated that the uptake of potassium was influenced by the intensity of light.

The data of this paper generally tend to show that light, regulating the general growth, also exercised an influence upon the rate of uptake of potassium.

Other effects of potassium upon the general metabolism of fruit trees have been studied by Wallace (35). A lack of potassium was shown by pathological symptoms in the leaves and by the quality of the fruit.

Briefly stated, the evidence as a whole indicates that potassium deficiency may diminish carbohydrate assimilation, protein synthesis, and the production of dry matter. A relationship between growth rate and potassium content exists in certain phases of development of some species. Species, and even varieties, show different degrees of susceptibility to potassium starvation. Generally, potassium accumulates in storage organs, but probably some of the potassium is free to circulate in the plant. No constant relationship between potassium and the dry weights of translocated compounds has been observed; potassium probably functions as a catalyst, but when under certain conditions of light the rate of utilization of carbohydrate is low and the rate of translocation rapid, a decrease in the total dry matter translocated may result from potassium deficiency. To a large extent, as judged by external morphology, sodium may replace potassium, but in certain species plants grown with little potassium are prone to suffer from a water strain.

## 4. SUMMARY.

This paper reports the results of experiments at Wisley, designed to study the influence of the daily period of light and the supply of available potassium upon the rate of accumulation of carbohydrates in the storage organs of *Phaseolus multiflorus*, *Dahlia* (2 varieties), and *Stachys tuberosa*.

1. The rate of accumulation of carbohydrates in the roots and tubers was governed by the length of the period of illumination to which the plants were exposed. Very short periods (of 5 hours' duration) produced small plants with small tubers. Short periods (of 10 or 12 hours' duration) of daily illumination caused a very high proportion of the total dry matter to be translocated rapidly to tuber and root. With longer periods of natural daylight, and with periods of 10 or 12 hours supplemented by 5 or 7 hours of electric light of weak intensity, the synthesized carbohydrates were utilized in upward growth. The development of the tubers was slower in comparison with that observed under periods of 10 and 12 hours' duration.

2. The influence of potassium was determined by growing the plants from seed, cuttings, or small pieces of tubers, of known potassium content, in sand to which was added known quantities of culture solutions either containing potassium or deficient in this respect. Sodium was used to replace potassium. Generally, the absence of potassium did not cause such pronounced differences in habit of growth as did the various light treatments.

3. Analyses of the tissues (at maturity) indicated that a dilution of the concentration of the potassium varying from three to ten times had been caused in the tissues.

4. Plants of *Stachys* deprived of an adequate supply of potassium were more susceptible to conditions causing wilting, both leaves and stems wilted, whilst plants receiving an equal amount of water, but with potassium, remained turgid.

5. A large reduction of the total dry weight occurred in dahlias as a result of deprivation of potassium.

6. The leaves of *Phaseolus multiflorus* afforded some scanty evidence, by means of starch tests, that the rate of formation and translocation of the starch was influenced by the amount of potassium available.

7. The stems of plants grown without potassium and subject to (12 hours or 10 hours) periods of daylight that caused rapid translocation to the tubers were found to contain very little potassium. The evidence indicated that the small quantity of potassium in the plant had passed to the developing tubers.

8. In all plants, potassium accumulated gradually in the tubers, moving in plants deprived of this element against the gradient, in other plants with the gradient of the concentration.



9. The replacement of potassium by sodium was without much *visible* effect upon the rate of formation of the tubers, but under short (10 or 12 hours) periods of daylight, when more rapid translocation to the roots or tubers took place, less dry matter passed to underground structures in plants deprived of potassium than in plants supplied with this element.

10. The circulation of at least part of the potassium in the plant must be entertained as a distinct possibility; neglecting the sodium present, the varying relationship between the weight of the potassium present in the entire plant and the weight of dry matter translocated appears to preclude any possible explanation of the process of translocation demanding a chemical combination of carbohydrate and metal (or metallic compound) in which the proportion of potassium is not extremely small; and from which the potassium cannot be readily released and used again. Potassium possibly functions as a catalyst facilitating condensation and hydrolysis of both starch and inulin. Sodium does not replace potassium completely.

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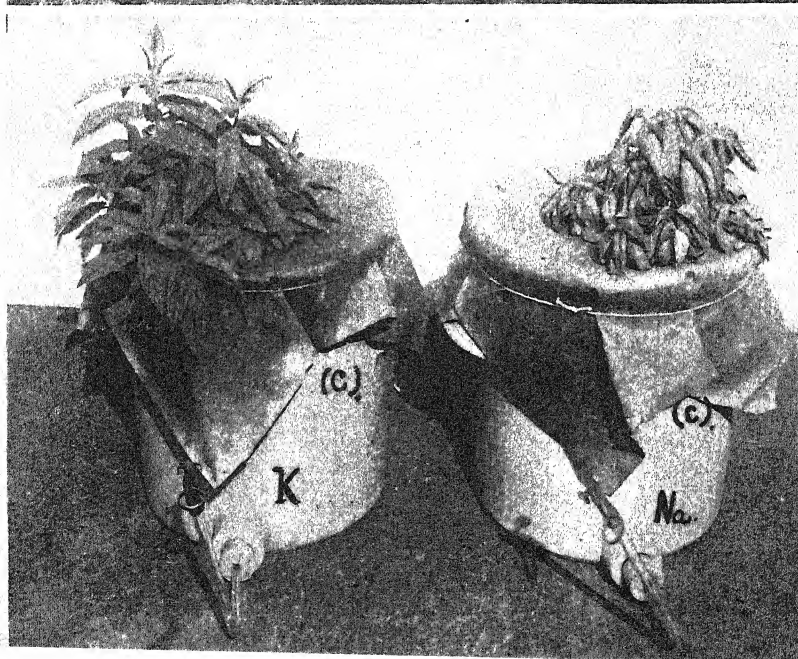
## EXPLANATION OF PLATE I.

Illustrating Mr. M. A. H. Tincker's and Dr. F. V. Darbishire's paper on 'Studies on the Formation of Tubers and Other Storage Organs'.

Fig. 1. Typical plants of *Stachys tuberosa*. B series, 10 hours' daylight and 5 hours' electric light. Grown with potassium and with sodium respectively. Photographs 5 p.m., July 23, to show wilting in plant grown with sodium. Equal previous watering.

Fig. 2. Typical plants of *Stachys tuberosa*. C series, 10 hours' daylight, grown with potassium and with sodium respectively. Photographed 5 p.m., July 23, to show wilting in plant grown with sodium. Equal previous watering.







# Observations on the Metabolism of Certain Sea-weeds.

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FOR some time past the attention of the authors has been attracted to the sea-weeds, and periodically they have made known certain facts about the metabolic products of these plants. On the present occasion the possible significance of these and other observations, hitherto unpublished, is considered.

The Phaeophyceae of Britain are zoned from the sublittoral through the littoral to the salt-marsh region, and accordingly do the conditions of life vary. At the lowest limit, the laminarias are emersed only at the neap spring tides; consequently they are exposed to the air for but short periods, and run but small risk of desiccation; they are subject to a narrow range of temperature, and to a wide range in light intensity. Thus, for the most part, they live in serene circumstances; almost a thalassic Avilion.

At the highest limit, the fucoids of the salt marsh are submerged only at high tides, maybe those only of the spring tides, *Pelvetia canaliculata* f. *libera*, for example. During their long exposure they may experience wide ranges in temperature, and the coincidences of weather conditions may produce such periods of drought that the plants may become almost brittle as a result of desiccation. Their existence is subaerial rather than aquatic.

The conditions of life in the littoral zone vary between these extremes, and need no further comment.

The Rhodophyceae, although for the most part sublittoral, also show some zonation; *Polysiphonia fastigiata*, for example, often is epiphytic on *Ascophyllum*, and *Bostrychia scorpioides*, a salt-marsh plant, often growing in association with *Pelvetia canaliculata* f. *libera*. The contemplation of

these extremes of existence leads to many questions, especially the correlation between metabolism and habitat. And in approaching these problems it is to be remembered that the fucoids in their metabolism diverge from the ways of the green plant in many things; this is indicated by the presence of laminarin presumably in place of starch; the, possibly, universal occurrence of mannitol; and the greater complexity of the cell-wall, as is shown by the presence of substances such as fucoidin, a carbohydrate ester of sulphuric acid, and algin, a polymerized mannuronic acid (Kylin (19), Cretscher and Nelson (8), and Bird and Haas (1)).

## FATS.

The accompanying table shows various fucoids arranged, as nearly as may be, in their order of zonation downwards: for the sake of comparison, two members of the Rhodophyceae, which normally occur at the extremes of zonation, are added, together with two Chlorophyceae which were collected at the highest tide levels. To avoid repetition, the total nitrogen and the relative presence of a biuret compound are given in addition to the amount of ether extract. The ether extract consists of fat and fat-soluble substances including pigments; the results for the most part are for gatherings made at the same season, although not necessarily, for obvious reasons, from the same locality.

Plant.	Ether extract.	Total N.	Biuret.
<i>Pelvetia canaliculata</i> f. <i>libera</i> . . . . .	8.62	1.02	++
<i>Pelvetia canaliculata</i> . . . . .	4.88	2.19	++
<i>Fucus vesiculosus</i> f. <i>volubilis</i> . . . . .	3.76	2.82	
<i>Ascophyllum nodosum</i> . . . . .	2.87		+
<i>Fucus vesiculosus</i> . . . . .	2.60	2.56	+
<i>Halydris siligiosa</i> . . . . .	2.18	1.34	very weak
<i>Himanthalia lorea</i> . . . . .	1.21	1.39	—
<i>Desmarestia aculeata</i> . . . . .	0.65	2.12	—
<i>Laminaria digitata</i> . . . . .	0.46	1.49	—
<i>Bostrychia scorpioides</i> . . . . .	0.31	2.84	—
<i>Chondrus crispus</i> . . . . .	0.204	2.33	—
<i>Enteromorpha intestinalis</i> . . . . .	0.217	{ 3.14 f. 4.34 s.	—
<i>Ulva latissima</i> . . . . .	0.185	3.32	—

Table showing the percentage of ether extract and total nitrogen in the dry weight of the weeds, and also the relative amount of biuret compound. The initials *f.* and *s.* against the values of total nitrogen of *Enteromorpha* indicate a fresh-water and a sea-water habitat respectively.

The amount of ether extract found in *Laminaria* corresponds pretty closely to that obtained by other investigators. Thus, König and Bettels (18) found 0.39 to 0.5 per cent. in the air-dried material, and



Hoagland (15), in his work on the kelps of the Pacific coast, gives the following percentages in terms of dry weight<sup>1</sup>:

<i>Laminaria Andersonii</i>	. . . .	0.65
<i>Macrocystis pyrifera</i>	. . . .	0.34-0.40
<i>Nereocystis Luetkeana</i>	. . . .	1.06
<i>Pelagophycus porra</i>	. . . .	0.27

Some seasonal variation, however, occurs, as is shown by the following figures:

<i>Pelvetia canaliculata</i>	. . . .	October,	4.88
"	. . . .	May,	5.84
<i>Laminaria digitata</i>	. . . .	March,	0.46
"	. . . .	July,	1.36

Such variation, however, was not found to occur in *P. canaliculata* f. *libera*, the average value for October being 8.62 per cent. and for May 8.65 per cent. This is hardly surprising, for the dominant factor in its life is periodic emersion, generally for relatively long periods.

The ether extract, although not uncommonly returned as fat, consists of true fat together with fat soluble substances, which may vary considerably in different plants. It was, therefore, thought desirable to find the amount of true fat, and also to ascertain the unsaponifiable residue and the degree of saturation as indicated by the iodine value. To this end typical plants of different levels of the littoral zone which could be obtained in quantity were selected together with the marsh form *libera*. Their analysis was done by Dr. B. Russell-Wells (28). The results of botanical interest are set out in the accompanying table, in which are shown (1) the average percentage of ether extract of the dry weed; (2) the percentage of true fat calculated from the fatty acids isolated from the ether extracts; (3) the percentage of unsaponifiable residue of the ether extract; (4) the percentage of fatty acid contained in the ether extract; (5) the iodine value of the ether extract; and (6) the iodine value of the fatty acids.

	1. Ether extr.	2. True fat.	3. U. res. of 1.	4. F. acid of 1.	5. Iodine V. of 1.	6. Iodine V. of 4.
<i>P. canaliculata</i> f. <i>libera</i>	8.0	6.2	7.6	72.5	106	107
<i>P. canaliculata</i> . . .	4.9	3.6	10.8	69.9	115	124
<i>F. vesiculosus</i> . . .	2.6	1.9	16.9	71.6	114	108
<i>L. digitata</i> . . .	0.3	0.16	25.9	49.9	123	110

The figures relating to the Phaeophyceae are of considerable interest in that they show a direct correlation between the amount of fat with the vertical distribution of the weeds, that is, with the duration of exposure. The greater the duration of emersion the greater is the exposure to

<sup>1</sup> No ether-extract values for genera corresponding to the other plants in our series have been found in the literature.

conditions favouring desiccation and to a wider range of temperature. Here there is a remarkable parallel with what may occur in land plants, for the chemical nature of the reserve food in many evergreen plants varies with the climatic conditions, and fat and fat-like substances may appear in the leaves on the advent of winter, often at the expense of starch. (Tuttle (33, 34), Meyer (33), Doyle and Clinch (9).) An important feature in the character of fats is their degree of saturation, and this for vegetable fats would appear to depend in no small degree on the conditions of growth. Of these conditions temperature is all important, which may be indicated by two examples. Ivanow (16) found that the cultivation of *Linum usitatissimum* in southern Russia promoted the formation of oleic acid fats in the seeds, whilst the seeds of plants grown in northern Russia had fats in which linoleic acid preponderated. In other words, the fats of seeds grown in hotter climates are more saturated than those grown in colder. This fact also is clearly demonstrated by the observations of Pearson and Raper (26), who grew pure strains of *Aspergillus niger* and *Rhizopus nigricans* on a culture medium at different temperatures. It was found that the degree of saturation of the metabolized fats, as indicated by the iodine values, varied with the temperature at which the cultures were maintained; the higher the temperature, the greater the degree of saturation.

When compared with the sea-weeds it is seen that the iodine value of the ether extract of the Phaeophyceae falls with the degree of emersion, being highest in *Laminaria* and lowest in *P. canaliculata*, f. *libera*, which means that the fat and fat-like substances of the latter plant are the more highly saturated, and this may be correlated with the more extreme conditions of its life, more especially a higher temperature for the most part of the year, and its prolonged periods of possible desiccation.

It is, however, to be pointed out that the iodine values of *P. canaliculata* and *F. vesiculosus* are practically identical, but the measure of their vertical separation is nothing more than a few feet.

This relation between the degree of saturation and the vertical distribution would not appear to obtain when the iodine values of the fatty acids of the selected plants are compared. It is uncertain what significance can be attached to these figures, for during the isolation of the fatty acids from the original ether extracts they became more saturated, although all reasonable precautions were taken. For this reason stress is not laid on the values obtained. Another feature of interest is the marked increase of the unsaponifiable residue with the relative degree of immersion.

For the rest, it may be mentioned that there is a greater proportion of liquid fatty acids in *P. canaliculata* f. *libera* than in *L. digitata*.

These observations apply only to the Phaeophyceae: the two Rhodophyceae examined, *Bostrychia* and *Chondrus*, representing the extremes of the habitat, contain but little fat, and the disparity between the amounts is

nothing like so great as in representative Phaeophyceae. Similarly, the two great Algae, *Enteromorpha* and *Ulva*, characteristic of the upper tidal reaches, contain but little fat, and there is no corresponding plant of the lower tidal limits with which to institute a comparison.

#### SUGAR AND SUGAR ALCOHOLS.

Some fundamental differences in the metabolism of the Phaeophyceae, as compared with the ordinary green plant, have been alluded to on an earlier page. The realization that the life of *P. canaliculata* f. *libera* is subaerial rather than aquatic suggested the question whether it diverged in its metabolism from its relatives and approached the green plant. The first and obvious investigation was the search for free sugars, typical products of the photosynthesizing green plant, for the earlier work of other investigators indicate the absence of free sugars or else their presence in minute quantities. Thus Hoagland (15) found no sugar in the various kelps of the Pacific coast, but Kylin (21), on the other hand, found traces of glucose. Our examination (Haas and Hill (10)) showed the presence of a small amount of free pentose, 0.018 per cent. of the wet weight of the weed, in *P. canaliculata* f. *libera*; some also was found in *P. canaliculata*, *Fucus serratus*, and *Ascophyllum nodosum*, but so small in amount that the titration figures were hardly significant. Also in *Pelvetia* there was found a small amount of a pentose complex, probably a disaccharide, which only reduced Fehling's solution after hydrolysis. Later publications have indicated the same absence or paucity of free reducing sugar, thus Colin and Ricard (7) found no determinable amount of reducing sugar in some fifteen fucoids, and Ricard (27) found neither glucose nor fucose in a free state in *Laminaria flexicaulis* and *L. saccharina*. Ricard (27) ascribes the glucose found by Kylin to the partial hydrolysis of laminarin during the preparation of the material. This absence, or extreme paucity, of free sugar is certainly curious, and perhaps finds a compensation in the presence of sugar alcohols.

Of the sugar alcohols which occur in the plant, the present occasion is devoted to the three hexahydric alcohols mannitol, sorbitol, and dulcitol. Of these, mannitol is the most common and will be considered first.

The sporadic occurrence of mannitol in many angiosperms of diverse affinity, in a few vascular cryptogams, in various agarics and moulds, and in lichens, for long has been a botanical curiosity. In the Phaeophyceae, however, the accumulation of evidence (Stenhouse (11), Colin and Ricard (7), Kylin (20, 22), and Segers-Laureys (29)) indicates its universal occurrence, although sometimes masked by the presence of its anhydrides, those rather ill-defined mannitans, as in *P. canaliculata* and its marsh form *libera* (Haas and Hill (11)). This suggests that in this phylum mannitol has

a definite metabolic rôle rather than being merely due to the inevitable consequence of a reaction of specific peculiarity.

That mannitol has a nutritive value in the metabolism of various moulds has long been known, but its precise value is dependent on various factors such as the hydrogen-ion concentration, and the nature of the nitrogenous constituent of the pabulum. Earlier work has shown that in the cultivation of various moulds, sugar has a higher nutritive value than mannitol (Went (35)), and the investigations of Obaton (25) indicate that *Sterigmatocystis nigra* grown in a culture medium containing both sugar and mannitol does not use the alcohol until the sugar has been consumed.

Within the mycelium mannitol is often associated with trehalose, and their relative amounts during growth was regarded as showing that the alcohol had its origin from the sugar. Thus Bourquelot (5), from his observations on various agarics, concluded that the trehalose is hydrolysed to glucose, which is then reduced to mannitol. On the other hand, Obaton found that the occurrence of mannitol in *Sterigmatocystis* is dependent on the presence of sucrose in the nutrient medium, if the sugar be abundant there will be much mannitol in the mycelium. He considers that the trehalose is formed from the mannitol, the process being governed by the hydrogen-ion concentration of the medium, being greatest at neutrality or slight alkalinity.

This association of trehalose and mannitol is not uncommon in the fungi, but the presence of one does not include the presence of the other. Thus *Penicillium glaucum* contains mannitol, but no trehalose; this also is true for *Agaricus campestris*, whilst the reverse occurs in *A. muscarius* (Müntz (16)).

It appears, however, that mannitol is not always associated with trehalose; thus Obaton found that the mannitol in *Apium graveolens*, the celery, is associated with sucrose when photosynthesis is very active.

Reference may now be made to the formation of mannitol by fermentative processes. It is common knowledge amongst wine makers that bacterial activity converts the fructose of grape juice into mannitol; Busolt (6) found that neither the fresh sap of *Phaseolus vulgaris* contained mannitol, nor the sterilized sap on keeping, but the unsterilized sap developed a considerable amount with the lapse of time. This observation is paralleled by that of Tutin (32), who found that the bacillus responsible for 'cider sickness' reduced the sugar of the apple juice to mannitol.

Turning to the fermentative activity of the moulds, Bourquelot (4) found mannitol to occur in the mycelium of *Aspergillus*; Birkinshaw, Charles, Hetherington, and Raistrick (2) identified it in the culture medium, containing glucose, on which various species and strains of *Aspergillus* had been grown. The amount of mannitol thus produced varied, but when aeration was restricted the amount of mannitol formed, sometimes as much

as 50 per cent. of the glucose fermented, was much greater than when there was free access to air; an important observation.

Birkinshaw and Raistrick (3) also found that glucose was fermented to mannitol and other products by *Helminthosporium geniculatum*, *Clasterosporium* sp., *Aspergillus Wentii*, and *Penicillium chrysogenum*.

The rarer sugar alcohols, dulcitol and sorbitol isomeric with mannitol, may be considered together. Dulcitol occurs in species of *Euonymus* and *Melampyrum*, whilst sorbitol is found in the fruits of *Pyrus aucuparia*, the apple and the pear. Sorbitol also is produced by the bacterial fermentation of apple juice (Tutin (31)).

Kylin examined a number of Rhodophyceae for mannitol and found none; like negative results attended our examination of *Batrachospermum moniliforme*, *Cystoclonium purpureum*, *Chylocladia articulata*, *Corallina officinalis*, *Gigartina stellata*, *Laurentia pinnatifida*, *Polysiphonia fastigiata*, and *Rhodymenia palmata*.

The fact that the mannitol of *P. canaliculata* f. *libera* is masked by the presence of mannitan prompted the examination of the red sea-weed *Bostrychia scorpioides*.

*Bostrychia* flourishes on the salt marsh at Blakeney Point, Norfolk, and grows in association with *Salicornia herbacea* and *Suaeda maritima*, and not uncommonly with *Pelvetia canaliculata* f. *libera*. It is free growing and forms a loose felt, sometimes lightly bedded in the muddy soil, or retained in position by the dense growth of *Salicornia* and *Suaeda*: its circumstances are therefore those of *P. canaliculata* f. *libera* and are subaerial rather than aquatic. Its examination revealed, not the presence of mannitol, but the presence of dulcitol and of sorbitol (Haas and Hill (12, 14)).

The consideration of these facts leads to no definite conclusions, but a few comments may be made.

With regard to the absence or paucity of free sugars, the conditions of life of the fucoids are hardly ideal for photosynthesis, since all-important factors, such as light, temperature, and exposure, are rhythmically limiting. The recent work of Ricard (27), for example, shows that in *Laminaria* the greatest amount of carbonaceous foodstuffs, mannitol and laminarin, occurred in the late summer and coincided with a period of high insolation, and incidentally of high temperature. Further, the thickness of the cell-walls of many of these plants is an impediment to the rapid diffusion of carbon dioxide into the plant. For these reasons carbon assimilation presumably is slow and sugars, if formed in the earlier stages, are immediately converted into other substances such as sugar alcohols and polysaccharides. An alternative hypothesis is that the sugar is of secondary origin and that mannitol, not sugar, is a primary photosynthetic product, which implies that the carbon metabolism of the fucoids is based on alcohol rather than

on sugar, and that the sugar may be an oxidation product of the alcohol. The problem is thus the relationship between the sugar and the alcohol. Some of the facts relating to the higher plants and the fungi, outlined on a previous page, do not help, but the work of Tutin, Raistrick, and others strongly indicates that in the fermentative processes studied mannitol has its origin from the sugar. In the works referred to mannitol is associated with either sucrose, trehalose, or glucose, all of which are hexoses; but in the fucoids the only free sugar definitely established is a pentose.

One other point may be commented on. Raistrick and his fellow workers found that a restricted supply of oxygen led to a great increase in the amount of mannitol formed: it is possible that this may be significant in the formation of this alcohol in some of the fucoids, for many of them are of a fleshy habit which connotes a slow gaseous diffusion. And in this connexion it is interesting to note that the occurrence of mannitol in other plants is not infrequently associated with a massive structure; *Opuntia*, the fleshy fruits of the olive and pineapple, carrot, cauliflower, various agarics, and lichens may be mentioned. The implication of the comparison must not, however, be unduly emphasized.

With regard to the presence of dulcitol and sorbitol in *Bostrychia* little can be written with profit, for the apparent absence of sugar alcohols from other red sea-weeds indicates that *Bostrychia* in this respect has a specific metabolism; something more than the conditions of the habitat is operative since *Polysiphonia*, growing epiphytically on *Ascophyllum*, contains no sugar alcohol, although *Ascophyllum* itself contains much mannitol. Further, an impeded gaseous diffusion would not appear to be a factor since *Bostrychia* with its delicately branched and slender thallus presents a relatively extensive surface.

The balance of evidence is in favour of the view that the sugar alcohols of the fucoids are secondary products derived from sugar. The absence or paucity of free sugars may be regarded as being due to the rate of their utilization approximating their rate of formation.

If this contention be right, the conversion of the sugar to the alcohol must be effected either by a definite enzyme or by 'protoplasmic' activity. Our experiments so far have failed to demonstrate a specific enzyme, but the matter is still being investigated.

#### NITROGEN METABOLISM.

In the preliminary work on the fucoids the total nitrogen was estimated as a matter of course; the results obtained are set forth in the first table on p. 56. The first estimations showed that the amount of nitrogen varied inversely with the fat, thus suggesting an inter-relation between the two, not an uncommon relationship in animal physiology. But further

analyses indicated that, as expected, the total nitrogen varied with the circumstances of the environment. In like manner the analysis of the distribution of nitrogen in its various combinations showed a marked variation, as is shown by the following figures relating to *Pelvetia*, *Fucus*, and *Laminaria*, in which the total nitrogen is expressed in terms of the dry weight of the weed and the various fractions in terms of the total nitrogen.

	Coll.	NH <sub>3</sub> .	Amide.	Amino.	Sol. N.	Total N.
<i>P. canaliculata</i> f. <i>libera</i>	Oct.	0.0	9.66	4.37	28.80	1.52
" "	July	0.0	9.73	5.04	26.40	1.08
<i>P. canaliculata</i> . .	April	0.0	18.59	13.66	43.79	2.8
" . .	July	0.0	10.67	6.46	30.77	1.13
<i>F. vesiculosus</i> . .	April	6.19	16.36	21.80	44.09	2.2
<i>L. digitata</i> . . .	Jan.	4.86	2.33	17.99	33.06	2.7

These results led to a rough field experiment with *P. canaliculata* and its form *libera*; a sufficiency of each were collected immediately prior to a high-tide epoch in July and placed in a depression of a shingle bank into which the high tides flowed and very slowly drained away. The plants were thus immersed in a bath for a considerably longer period than the exposed plants of the nearby marsh.

The following table gives the results:

		NH <sub>3</sub> .	Amide.	Amino.	Sol. N.	Total N.
<i>P. canaliculata</i> f. <i>libera</i>	Bathed	4.93	1.58	15.19	24.29	1.05
" "	Exposed	0.0	9.73	5.04	26.40	1.08
<i>P. canaliculata</i> . .	Bathed	6.49	5.09	8.63	32.29	1.22
" . .	Exposed	0.0	10.67	6.46	30.77	1.33

The results given in these two tables may be summarized:

(1) The presence of ammonia in all plants except in the exposed plants of *P. canaliculata* and its form *libera*.

(2) There is a considerable range in the amounts of amide and amino nitrogen, but in the exposed plants of the high zones (*P. canaliculata* and the form *libera*) the amino nitrogen is always less than the amide nitrogen, whilst in the bathed plants of these two weeds and in *Fucus* and *Laminaria*, which are naturally covered by water for longer periods, the amino nitrogen is greater than the amide nitrogen.

(3) The amount of total nitrogen is lowest in the summer months, but the number of seasonal analyses are too few to warrant any definite conclusion.

The occurrence of trimethylamine also was established in *P. canaliculata* and other plants, a fact which confirms the earlier observations on *Fucus vesiculosus* and *F. serratus* by Kapeller-Adler and Csató (17).

These analyses do not lead to any generalization, although indications

are not wanting that the period of emersion has its effect on the nitrogen metabolism. Indeed, it quickly became obvious that elucidation on many of the points raised was only possible by carefully controlled cultures on an adequate scale. For this reason the analyses were discontinued until such controlled growths are possible.

In our earlier work, the search for sugar, it was frequently noticed that on testing concentrated extracts of the weeds for sugar the Fehling's solution acquired a characteristic mauve colour, the biuret reaction. This clue was followed, and ultimately the presence of an octapeptide of glutamic acid was demonstrated (Haas and Hill (13)). The examination of other fucoids naturally followed with the result, set out in the table on p. 56, that only those of the higher regions of the littoral zone were found to give positive results, and these were strongest in *P. canaliculata* and its form *libera*, which are the highest in the series.

The only other polypeptide of normal occurrence in the plant is the tripeptide glutathione,<sup>1</sup> although the amides asparagine and glutamine, which also give the biuret reaction, may occur when there is a deficiency of carbohydrate, as in etiolated seedlings of certain plants, or as translocatory products of proteins.

Polypeptides normally are stages in the synthesis and analysis of proteins: it is thus of considerable interest to find a representative of them apparently a constant component of the cell-sap; for once its presence has been demonstrated, it has always been found, no matter at what period of the year the plants had been collected, although there is a seasonal variation, it being more abundant in February and September than in July. But notwithstanding this, the presence of the polypeptide in the fucoids is not unlikely due to the periods of emersion and the consequent interruption of normal metabolism, for its amount falls off with lessening emersion and it is absent from the fucoids of the lower zones. This view is supported by the following observations.

The amount of the octapeptide in *P. canaliculata* f. *libera* immersed during a period of high tides in the low shingle mentioned above was compared with that contained in the plants exposed on the adjacent marsh during the same period. A colorimetric examination showed that the exposed plants contained much more than the bathed plants. In order to put the subject on a more definite basis, the mercury salt of the polypeptide was prepared, and the weight of the mercury compound obtained was calculated on the dry weight of the weed. The result was that the exposed plants contained 7.3 per cent. more polypeptide than the more or less continuously bathed plants.

On another occasion, a comparable experiment was made in which the plants were continually submerged in a large shallow bath filled with sea-

<sup>1</sup> Its range in the vegetable kingdom has yet to be determined.



water, which was periodically renewed, during a high-tide cycle. A comparison of their polypeptide content with that of plants growing on the marsh immediately adjacent showed a 34.7 per cent. increase in favour of the more exposed plants.

Turning to the Rhodophyceae, the following plants were examined for a biuret reaction: *Batrachospermum moniliforme*, *Bostrychia scorpioides*, *Chondrus crispus*, *Chylocladia articulata*, *Cystoclonium purpureum*, *Delesseria ciliata*, *Gigartina stellata*, *Laurentia pinnatifida*, *Polysiphonia fastigiata*, and *Rhodomenia palmata*. All gave negative results, but *Corallina officinalis*, *Lithothamnion incrustans*, and *Griffithsia flosculosa* gave a positive reaction, and subsequent analysis showed that the biuret reaction of *Corallina* is due to a pentapeptide.<sup>1</sup> The hypothesis adopted to explain the presence of a peptide in the brown algae also is here applicable. In the fucoids carbon assimilation is depressed owing to the periods, often prolonged, of emersion, consequently there is not enough sugar generally available to combine with the intermediate products of nitrogen assimilation, which products therefore accumulate. *Corallina* and *Lithothamnion* live just about the *Laminaria* zone and therefore are exposed only at the neap tides, so that any depression in carbon assimilation must be due to causes other than exposure. The cause is not far to seek: *Corallina* and *Lithothamnion* grow attached to rocks and often in close company with other weeds; further, they are ensheathed with a not inconsiderable coating of calcium carbonate.<sup>2</sup>

On the other hand, *Griffithsia* is not encrusted, but it grows below the *Laminaria* zone in shaded situations. Thus it would appear in these three plants that light is limiting, and it is this factor which depresses carbon assimilation below the rate of possible protein synthesis; there is thus an accumulation of peptide.

#### SUMMARY.

1. This paper is a commentary on certain facts relating to the metabolism of marine algae.

2. The facts considered relate to:

- (a) The direct increase of the fats and fat-like substances with the degree of emergence; the increase in the unsaponifiable residue with the depth of immersion; and the increase in the saturation of the ether-extract with the degree of emergence.
- (b) The absence or paucity of free sugars; the probably constant

<sup>1</sup> This observation was made when this paper was almost completed. The investigation of the peptide is being continued.

<sup>2</sup> Dr. Russell-Wells finds that the amount of calcium carbonate in *Corallina* is, on the average, 80.89 per cent. of the dry weight of the weed.

occurrence of mannitol in the Phaeophyceae; the absence of mannitol, but the presence of dulcitol and sorbitol in one member of the Rhodophyceae, namely *Bostrychia*.

- (c) Nitrogen metabolism, especially the occurrence of an octapeptide of glutamic acid in the Phaeophyceae of the higher zones, and the presence of a pentapeptide in three members of the Rhodophyceae, namely *Corallina officinalis*, *Lithothamnion incrustans*, and *Griffithsia flosculosa*.

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# The Relation between Water-content, Chlorophyll-content, and the Rate of Photosynthesis in Some Tropical Plants at Different Temperatures.

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With six Figures in the Text.

## INTRODUCTION.

A FRESH aspect of research work on the physiology of photosynthesis is opened by the recent contribution by Dastur (3 and 4). He has shown that the water-content of a leaf is one of the important internal factors on which the photosynthetic activities of leaves depend; a fact which, previous to his work, was not taken into account when the rate of photosynthesis of the leaves of various plants was determined by various workers. Whether it is a factor which affects the rate of photosynthesis directly or indirectly is at present not clear, though the results leave no doubt of its importance.

A further extension of Dastur's work seemed advisable, since he worked at one temperature, and the carbon dioxide of the air was a limiting factor in his experiments. It was of interest to determine the influence of the water-content at different temperatures and when the carbon dioxide is supplied in excess. The results obtained with such external conditions would be of great value in estimating the influences of water-content on the rate of photosynthesis. If the rate of photosynthesis of a leaf at different temperatures is related to its water-content when the other external factors are in excess, it would furnish additional proof of the importance of water-content in the process.

*Note:* In all figures the relation between the water-content and photosynthesis and the relation between the chlorophyll-content and photosynthesis are shown as Assimilation Numbers *W* and Assimilation Numbers *CH* respectively, circles indicating the former and crosses the latter.

Dastur and Buhariwala (5) have recently devised an accurate spectrographic method of estimating the chlorophyll-content of a leaf. With this valuable method now available, it should also be possible to determine the chlorophyll-content of a leaf whose photosynthetic activity is measured. In this way two internal factors, water-content and chlorophyll-content, could be accurately determined, and they could be compared side by side with the rate of photosynthesis of the same leaf. This has not hitherto been done. Willstatter and Stoll (23) and other workers determined the chlorophyll-content of a number of leaves whose rate of photosynthesis was measured in each experiment and they failed to find any relationship between the two. They did not take into account the water-content of the leaves. A number of detached leaves in each experiment introduces an error, as the leaves, apparently of the same age, but obtained from different branches, have different water-content and chlorophyll-content, as shown by Dastur and Buhariwala (5). No two leaves, even of the same age, would be exactly alike in these respects. It would, therefore, be of interest to investigate by the methods devised by Dastur (4) and Dastur and Buhariwala (5) the rate of photosynthesis, and the chlorophyll-content of a single leaf, and so determine whether the rate of photosynthesis depends upon the water-content or the chlorophyll-content of a leaf, or upon both.

Dastur (4) established the relations between the water-content and the rate of photosynthesis of some temperate plants. This investigation would also help to determine whether similar relationships between the water-content and the rate of photosynthesis holds in the case of the tropical plants.

A large amount of work has been done on the effect of temperature on the rate of photosynthesis with a view to determine whether the photosynthesis is purely a photochemical process, or whether any other chemical process is involved in it. This has been done by determining the temperature coefficient  $Q_{10}$  for the process. The temperature coefficient should obey the van't Hoff's rule and not exceed 1.4 if it is purely a photochemical process, and it should be of a higher value than 1.4 if some purely chemical process is involved in it. It has been shown by various workers like Leitch (13) and others that many processes in plant organism obey van't Hoff's rule, such as respiration and growth. Much of the previous work done to determine the temperature coefficient of the photosynthetic process is open to objections: (1) only apparent assimilation was measured, and in some cases like Kreuzler's (10, 11, 12) work was done with unhealthy material. Mathaei (15) and Blackman and Mathaei (2) by carrying on a series of researches have greatly advanced our knowledge on the influence of temperature on photosynthesis when no other external factor such as light or carbon dioxide is limiting the rate of the process. They came to

the conclusion that between 5° C. and 20° C. the rate of photosynthesis remained constant, and when no other factors were limiting, the values obtained indicated that between these temperatures the van't Hoff's rule was obeyed,  $Q_{10}$  being 2.1 in the case of Cherry Laurel leaves and  $Q_{10}$  being 2.5 in the case of *Helianthus tuberosus*. Van Amstel (19) determined the temperature coefficient for *Elodea* and found it to be 1.26, but her results can hardly be relied upon on account of various defects in her work. Haas and Osterhurst (6) have obtained the same values for temperature coefficients for photosynthesis in *Ulva rigida*, and so also Warburg (20, 21) in *Chlorella*. It is evident from the above that the photosynthetic process obeys van't Hoff's Rule between 5° C. and 25° C., while above 25° C. the rate of photosynthesis falls off with time.

The facts mentioned above indicate that the optimum temperature for photosynthesis is 25° C. for the plants in the temperate regions. It would be of interest to determine the optimum temperature for photosynthesis in the case of tropical plants as the normal temperature of the air is in the neighbourhood of 30° C. No such observations are recorded for such plants, except those made by McLean for coco-nut leaves, and by Yap (24) for sugar-cane leaves. But both the workers determined the rate of photosynthesis at ordinary temperature of the air. It is not attempted here to determine the temperature coefficient on account of the difficulty of working with lower temperatures than 20° C.

#### METHOD.

The apparatus devised by Dastur was used in this investigation with certain modifications.

During the course of this investigation potted plants were used. A leaf of about the same age, as determined from its numerical position from the apex, was enclosed in the leaf chamber in all experiments, and care was taken to select leaves of healthy appearance. The temperature of the chamber was controlled to 0.5° C. To this end, one new device was introduced. It consisted of a simple metallic drum with an outlet enclosed in a jacket to prevent heat losses due to radiation. Water at suitable temperature, as was found necessary for each experiment, was kept in it and was allowed to flow to the leaf chamber. Previous to starting the experiment the flow was regulated and the constant temperature was each time assured. In addition to this, a lead coil surrounded by salt and ice in a wooden box was also used for working at lower temperatures. The source of light was a gas-filled Osram lamp (220 volts; 1,500 watts) kept a distance of 32 cm. from the leaf.

An air mixture containing 5 per cent. of carbon dioxide was passed through the leaf chamber at the rate of one litre per hour. The gas current,

after passing through the washer, was divided before it reached the leaf chamber. One of the divided streams of air mixture passed directly to the absorption apparatus. The second stream of the air mixture passed through the leaf chamber, and then passed through another absorption apparatus. In this way the carbon-dioxide concentration of the air mixture was determined both before and after it had passed through the leaf chamber. The difference in the carbon-dioxide concentration in the air mixture gave the amount of carbon dioxide absorbed by the air. This value gives 'apparent assimilation'. To obtain a value for 'real assimilation' the amount of carbon dioxide respired by the leaf during the same period and at the same temperature for which the carbon-dioxide absorption is measured, is added to the value of 'apparent assimilation'. In order to free the chamber and the drying apparatus of carbon dioxide before respiration experiments, it was found necessary to pass air through sodium hydroxide kept in a stoppered U-tube connected with both the sets between the calcium-chloride tube and the sulphuric-acid tower. The latter was also freed of carbon dioxide at that time.

The leaf was detached after taking four readings as above, weighed immediately, and its total area determined on graph paper. It was then dried in a vacuum desiccator containing calcium chloride, and its weight when dry was determined. The difference in the weight, when fresh and when dried, was taken as its water-content. The leaf was then powdered and chlorophyll extracted in pure condition, according to the method devised by Willstatter and Stoll (22).

A pure solution of chlorophyll in sulphuric ether was obtained, and its concentration determined according to the method devised by Dastur and Buhariwala (5). The 'real assimilation' value, water-content, and the chlorophyll-content of the leaf thus obtained were reduced to 100 square cm. of leaf area. Care was taken to ensure uniform conditions for all experiments. Over and above this, all precautions were taken as indicated by Dastur (4). On an average, it took four days to complete one experiment.

A total number of about 120 such experiments were performed from July 1928 to January 1930.

Dastur (4) has expressed his results by plotting the real value of assimilation per unit area against the water-content for the same area, and has shown that the assimilation value at 20° C. increases as the water-content increases. But this method of expressing the results is not suitable when the readings are taken at different temperatures, and the increasing temperature has an effect upon the rate of assimilation of a leaf. So it is not expected, as the results below show, to obtain a correlation between the water-content and carbon-dioxide assimilation of a leaf as obtained by Dastur (4). This would only be true if the assimilation values are determined at a fixed temperature (vide Table XVII).



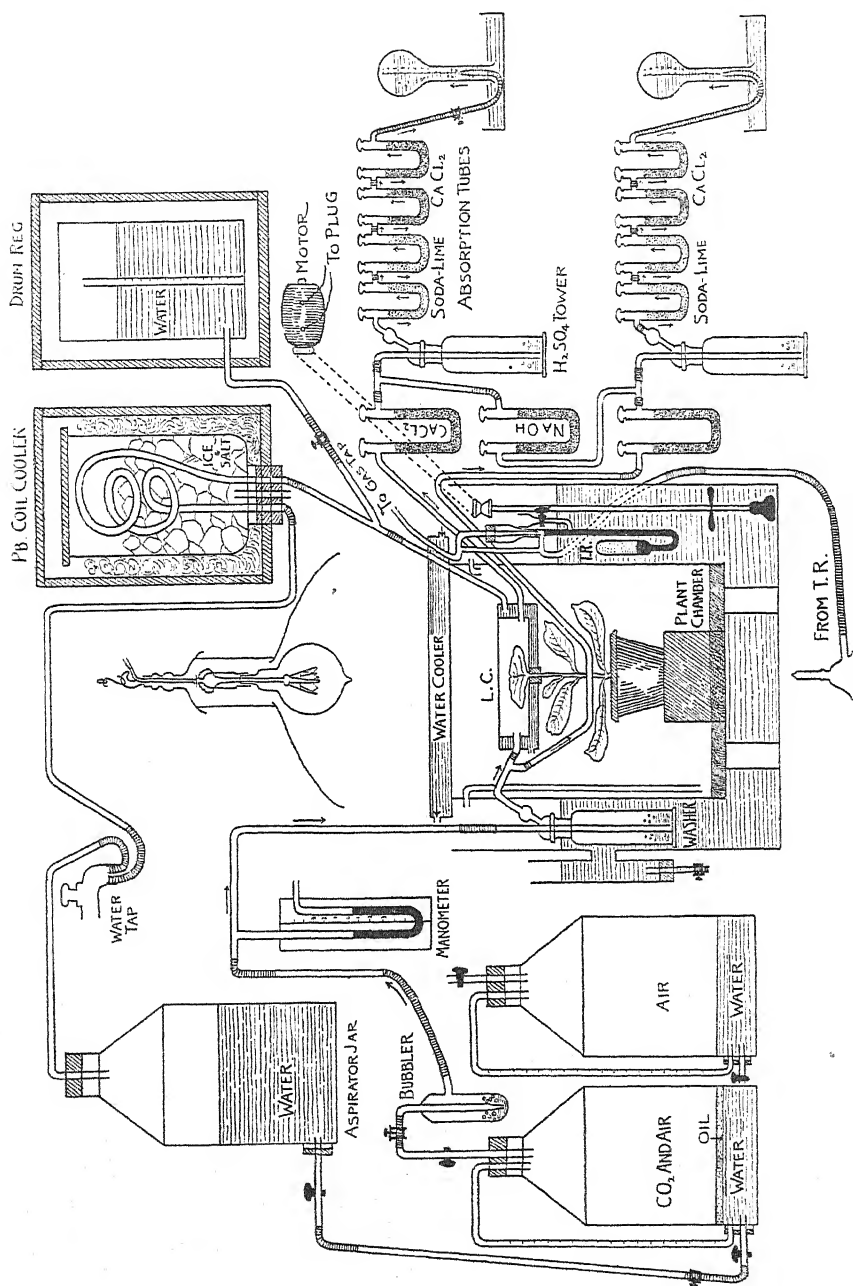


FIG. 1. Diagram of the apparatus in longitudinal section. ( $\frac{1}{8}$  nat. size.)

TABLE I.  
*Abutilon asiaticum* G. Don.

Leaf no.	Temp. °C.	CO <sub>2</sub> assim. per hour per sq. dm. leaf area.	Water-content per sq. dm. leaf area.
		gm.	gm.
31	30	0.0178	0.5315
32	31	0.0220	0.5950
33	32	0.0250	0.6000
34	33	0.0286	0.5950
35	34	0.0240	0.5315
36	35	0.0182	0.6000
37	36	0.0132	0.5929
38	37	0.0078	0.6420

It is evident from the above results that the assimilation value per unit leaf area increases as the temperature is increased, and with further increase in temperature beyond 33° C. the rate of carbon-dioxide assimilation falls. The rise and fall in the rate of carbon-dioxide assimilation are very steep as the results clearly show. As the results stand it is not possible to estimate the part played by water-content in this process. It is clear that the water-content fluctuates from 0.5315 gm. to 0.6420 gm., and apparently bears no relation to the rate of carbon-dioxide assimilation.

Willstatter and Stoll (23) have expressed their results as 'assimilation numbers' in their researches on photosynthesis:—

$$\text{Assimilation number} = \frac{\text{CO}_2 \text{ assimilated in 1 hour by 1 sq. dm. of leaf area in gm.}}{\text{chlorophyll-content per 1 sq. dm. of leaf area in gm.}}$$

If the rate of photosynthesis depended upon the chlorophyll-content of a leaf, the assimilation number should be constant at constant temperature. This was not found to be the case, as they obtained very complex values of the assimilation number. Dastur (4) in expressing his results has not adopted this method of Willstatter (22) because it was, perhaps, not found necessary in order to show the relation between water-content and the rate of assimilation of a leaf. But in this investigation, on account of the difficulties of expressing the results in the same manner as done by Dastur (4), it was necessary to have recourse to the method adopted by Willstatter and Stoll (23). If the water-content of a leaf had any effect on its rate of assimilation, the assimilation numbers obtained by dividing carbon-dioxide assimilation per hour per one square decimetre of leaf area by the water-content of the same area should rise with the increasing temperatures. If the temperature is constant the assimilation numbers should also remain constant. In order to avoid confusion the assimilation number will be designated as assimilation number *W* to

distinguish it from the assimilation numbers of Willstatter and Stoll (23), which will be designated as assimilation number *CH*, as no suitable term to express the former suggested itself.

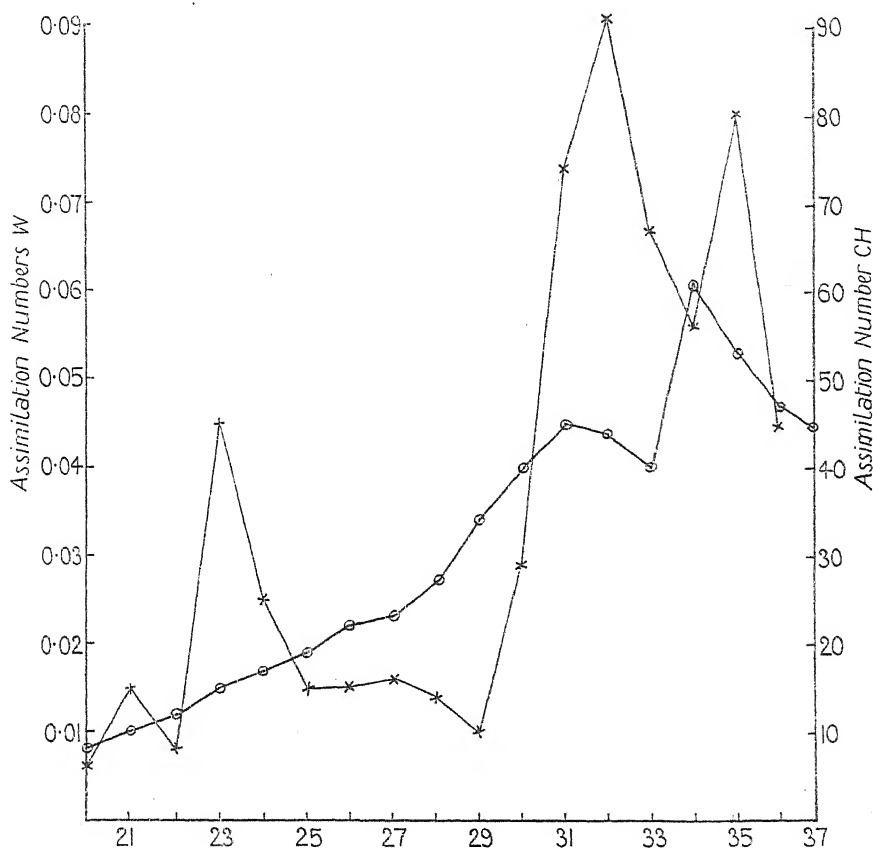


FIG. 2. Curves illustrating the relationship of water-content and of chlorophyll-content with photosynthesis in the leaves of *Abutilon asiaticum* G. Don., at different temperatures.

TABLE II.

*Abutilon asiaticum* G. Don.

Leaf No.	Temp. °C.	App. assim. per sq. dm. per hour.	Water-content per sq. dm. leaf area.	Assimilation No. <i>W</i> .
		gram.	gram.	
31	30	0.0178	0.5310	0.034
32	31	0.0220	0.5950	0.037
33	32	0.0250	0.6000	0.042
34	33	0.0286	0.5950	0.048
35	34	0.0240	0.5315	0.045
36	35	0.0182	0.6000	0.030
37	36	0.0132	0.5929	0.023
38	37	0.0078	0.6420	0.012

The results clearly indicate that the assimilation number  $W$  of a leaf increases as the temperature is increased. It reaches its maximum at  $33^{\circ}\text{C.}$ , and with further increases of temperature the rate of assimilation falls.  $33^{\circ}\text{C.}$  seems to be the optimum temperature when the rate of assimilation reaches its maximum and the other external factors do not limit the process.

The assimilation number  $W$  in Table II shows constant increase up to  $33^{\circ}\text{C.}$ , and then it begins to fall as the temperature rises, indicating that the rise in the rate of carbon-dioxide assimilation of a leaf with the increasing temperature is clearly related to its water-content, and so also the fall in its rate of assimilation as the temperature is increased above  $33^{\circ}\text{C.}$  If the water-content has no effect on the rate of photosynthesis this should not be the case, and there should not be such regular rise and fall in the assimilation numbers  $W$ . The rise and fall in the assimilation numbers are not constant with any rise of  $1^{\circ}\text{C.}$  in the temperature, and this could not be expected on account of the interaction of various internal factors, and as the experiments are carried out with different leaves. The water-content of one leaf is greater or less than that of another in the series, and this may cause great differences in the rates of assimilation.

For the reasons stated in the introduction, the chlorophyll-content of each leaf was determined according to the method devised by Dastur and Buhariwalla (5). This method has an advantage over that of Willstatter and Stoll (23) in that the chlorophyll-content of a single leaf can be directly and more accurately determined. The determinations of the chlorophyll-content enables us to determine the assimilation numbers ( $CH$ ) by Willstatter and Stoll (23), and to see whether they remain constant at one temperature and increase or decrease with the temperature. The two sets of assimilation numbers would then show which is more important in determining the rate of assimilation of a leaf. The three quantities in the leaves of *Ricinis communis* L. were therefore determined.

TABLE III.  
*Ricinis communis* L.

Leaf No.	Temp. $^{\circ}\text{C.}$	$\text{CO}_2$ assim. per hour per sq. dm. leaf area.	Chl. content per sq. dm. leaf area.	Water content per sq. dm. leaf area	Assimilation No. $CH$ .	Assimilation No. $W$ .
		gm.	gm.	gm.		
39	25	0.0088	0.00063	1.0220	12	0.009
40	26	0.0107	0.00062	1.0960	17	0.010
41	27	0.0129	0.00061	1.0745	10	0.011
42	28	0.0143	0.00073	1.0456	19	0.014
43	29	0.0159	0.00060	1.0860	29	0.015
44	30	0.0177	0.00064	1.0802	27	0.016
45	31	0.0181	0.00067	1.0910	27	0.017
46	32	0.0225	0.00070	1.1960	31	0.019

The range of temperature used in the above set was from 25° C. to 32° C. This was done purposely to see if the rate of assimilation increased continually as the temperature increased and so also the assimilation numbers *CH* and assimilation numbers *W*. In this case the assimilation number *W* continually increases, but the assimilation number *CH* shows small fluctuations.

TABLE IV.

*Abutilon asiaticum* G. Don.

Leaf No.	Temp. °C.	CO <sub>2</sub> assim. per hour per sq. dm. leaf area.	Chl. content per sq. dm. leaf area.	Water-content per sq. dm. leaf area.	Assimilation No. <i>CH</i> .	Assimilation No. <i>W</i> .
		gm.	gm.	gm.		
15	20	0·0059	0·00097	0·6862	6	0·008
16	21	0·0069	0·00044	0·6671	15	0·010
17	22	0·0083	0·00097	0·6862	8	0·012
18	23	0·0100	0·00022	0·6530	45	0·015
19	24	0·0115	0·00044	0·6671	25	0·017
20	25	0·0116	0·00074	0·5852	15	0·019
21	26	0·0135	0·00088	0·6045	15	0·022
22	27	0·0147	0·00097	0·6760	16	0·023
23	28	0·0162	0·00112	0·6274	14	0·027
24	29	0·0224	0·00222	0·6530	10	0·034
25	30	0·0257	0·00089	0·6045	29	0·040
2	31	0·0316	0·00049	0·702	74	0·045
3	32	0·0326	0·00039	0·744	91	0·044
4	33	0·0302	0·00038	0·704	67	0·040
5	34	0·0444	0·00086	0·733	56	0·061
6	35	0·0388	0·00051	0·729	80	0·053
7	36	0·0355	0·00083	0·744	45	0·047
8	37	0·0318	0·00032	0·724	103	0·044

TABLE V.

*Ricinus communis* L.

Leaf No.	Temp. °C.	CO <sub>2</sub> assim. per hour per sq. dm. leaf area	Chl. content per sq. dm. leaf area	Water-content per sq. dm. leaf area	Assimilation No. <i>CH</i> .	Assimilation No. <i>W</i> .
		gm.	gm.	gm.		
63	25	0·0195	0·00154	1·188	15·62	0·016
64	26	0·0178	0·00046	1·022	39	0·017
65	27	0·0201	0·00085	1·008	23	0·020
66	28	0·0246	0·00098	0·8835	25	0·027
67	29	0·0290	0·00062	1·011	47	0·029
68	30	0·0322	0·00071	1·020	45	0·032
69	31	0·0365	0·00112	1·093	32	0·033
70	32	0·0400	0·00125	1·113	32	0·036
71	33	0·0436	0·00094	0·9936	46	0·044
72	34	0·0486	0·00115	1·054	42	0·046
73	35	0·0504	0·00122	1·040	41	0·049
74	36	0·0541	0·00130	1·047	41	0·056
75	37	0·0399	0·00076	0·960	52	0·042

In order to support the results so far obtained a series of experiments was made with the leaves of *A. asiaticum* G. Don., and with those of *R. communis* L. The temperature was increased by one degree from 25° C. to 37° C. in the case of the leaves of *R. communis* L., and from 20° C. to 37° C. in the case of the leaves of *A. asiaticum* G. Don. The results obtained are shown in Tables IV and V.

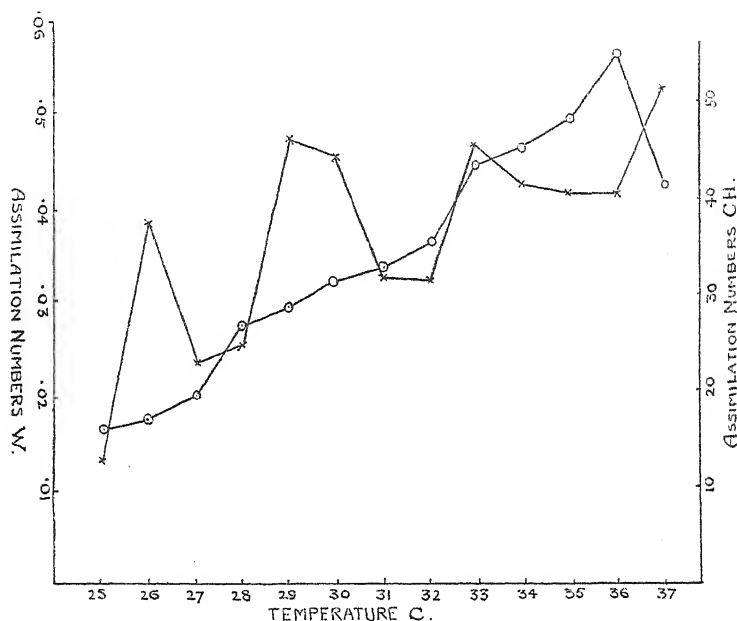


FIG. 3. Curves illustrating the relations of the water-content and of the chlorophyll-content with photosynthesis in the leaves of *Ricinis communis* L. at different temperatures.

The rate of CO<sub>2</sub> assimilation continually increases from 20° C. to 34° C. in the case of *A. asiaticum* G. Don., and then it begins to fall. The assimilation was 5.9 mg. at 20° C. per hour per one square decimetre of the leaf area, and it rose to 44 mg. at 34° C. The rate of assimilation then falls to 31 mg. at 37° C. The assimilation numbers *W* show the same behaviour (except in one experiment at 33° C.). The assimilation numbers *CH* do not show similar behaviour, and they show such great fluctuations that no conclusions could be derived from them (*see* Figs. 2 and 3).

The rate of assimilation in the leaves of *R. communis* L. was 19 mg. per hour per one square decimetre of the leaf area at 25° C. It rose steadily from 19 mg. for the same duration and leaf area at 36° C. The assimilation numbers *W* showed rise from 0.017 to 0.056, and then they fell to 0.042 at 37° C. The assimilation numbers *CH* do not show any regularity.

In order to get confirmation of the above results obtained with the leaves of *A. asiaticum* G. Don. and *R. communis* L. a set of experiments

was performed with the leaves of a garden variety (large-leaved) of *Helianthus annuus* L., commonly grown in Bombay in cold weather. The results obtained are given in Table VI.

TABLE VI.

*Helianthus annuus* L.

Leaf No.	Temp. °C.	CO <sub>2</sub> assim. per hour per sq. dm. leaf area.	Chl. content per sq. dm. leaf area.	Water-content per sq. dm. leaf area.	Assimilation No. CH.	Assimilation No. W.
		gram.	gram.	gram.		
99	31	0.0597	0.00080	1.624	74	0.037
100	32	0.0725	0.00078	1.893	93	0.036
101	33	0.0830	0.00090	1.844	92	0.044
102	34	0.0902	0.00088	1.832	102	0.049
103	35	0.0935	0.00099	2.078	94	0.050
104	36	0.1067	0.00111	2.076	96	0.053
105	37	0.0728	0.00119	2.189	61	0.035
106	38	0.0631	0.00097	1.890	65	0.029

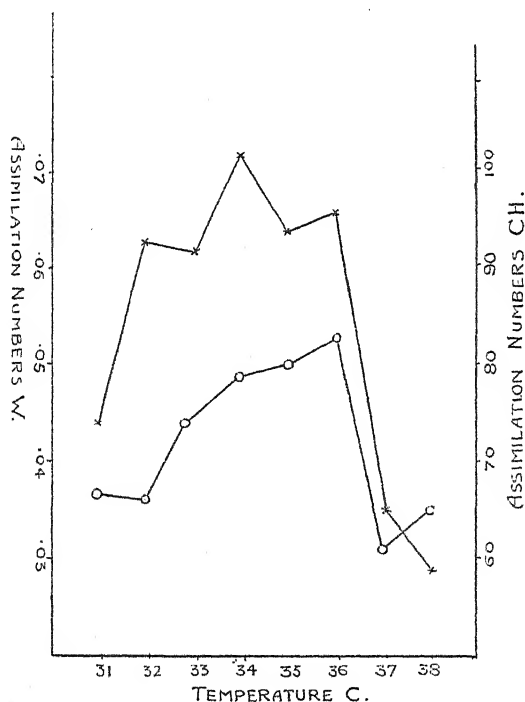


FIG. 4. Curves illustrating the relations of the water-content and of the chlorophyll-content with photosynthesis in the leaves of *Helianthus annuus* L. at different temperatures.

The results are very similar to those obtained for the other plants. The rate of assimilation is higher in these leaves than the rates of assimilation of the leaves of the other plants. The maximum rate of assimilation

is at 36° C., the leaf absorbing 106 mg. of carbon dioxide per hour per square decimetre of leaf area. The assimilation numbers *W* show a steady rise from 0.037 to 0.053, and then they begin to decline at 37° C. The assimilation numbers *CH* show marked fluctuations.

The results are shown graphically in Fig. 4. Experiments were also performed with the leaves of *Phaseolus vulgaris*.

After the results of five experiments were obtained the potted plants of *P. vulgaris* L. in the garden of the Institute all died suddenly and the whole series of experiments could not be completed. The results of the five complete experiments were similar to those obtained for the species, but as they are few they are not given.

In Tables I and II the temperature for the maximum rate of assimilation in the leaves of *A. asiaticum* G. Don. was found to be 33° C., while in Table IV the maximum rate of assimilation was reached at 34° C. with the same species. Thus there was a difference of one degree in the temperature at which assimilatory activity was the greatest. From the results of the two series of experiments performed with the same species it was evident that the rates of assimilation were not the same at the same temperature. This was due to the difference in the seasonal activities of the leaves at different times of the year. The plants had a higher activity in the monsoon months, from July to November, than that of the plants in the months from January to April. This was a point which could easily be tested ordinarily by Sach's iodine test. The leaves from the plants grown in the monsoon months showed greater abundance of starch than those grown in the winter months. The following table shows the marked difference in the rates of carbon-dioxide assimilation in the two seasons of the year.

TABLE VII.

*Abutilon asiaticum* G. Don.

Leaf No.	Temp. °C.	July to August.		December to February.		
		Assim. in one hour per sq. dm. leaf area.	Assimilation No. <i>W</i> .	Assim. in one hour per sq. dm. leaf area.	Assimilation No. <i>W</i> .	Leaf No.
		gm.		gm.		
25	30	0.0257	0.040	0.0178	0.034	31
2	31	0.0316	0.045	0.0220	0.037	32
3	32	0.0326	0.044	0.0250	0.042	33
4	33	0.0302	0.040	0.0286	0.048	34
5	34	0.0444	0.061	0.0240	0.045	35
6	35	0.0388	0.053	0.0182	0.030	36
7	36	0.0385	0.047	0.0132	0.023	37
8	37	0.0318	0.044	0.0078	0.012	38

The highest rate of assimilation per unit area in the monsoon months is 44 mg. per hour, and 28 mg. per hour in the winter months. Con-



sequently it is possible that the maximum point may be shifted to one degree higher. The seasonal variations of photosynthetic activity were also noticed in the case of the leaves of *R. communis* L., as Table VIII (p. 82) will show.

The rate of assimilation in the leaves of *R. communis* L. in the months of July–August is much higher than the rate of assimilation of the leaves in the winter months. The rate is nearly double at certain temperatures,

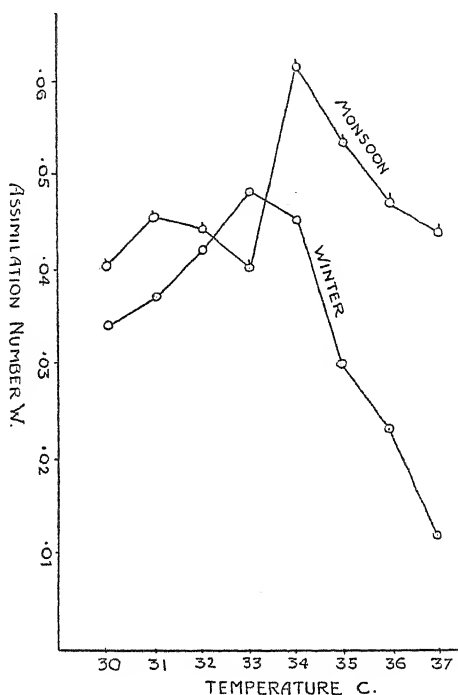


FIG. 5. Curves illustrating the differences in the seasonal photosynthetic activities of the leaves of *Abutilon asiaticum* G. Don. at different temperatures.

as at 25° C. and 29° C., or more than double. In the monsoon months it rises from 19.5 mg. to 40 mg. as the temperature is increased from 25° C. to 32° C. On the other hand, the rate of carbon-dioxide assimilation in winter rises from 8 mg. to 22.5 mg. within the same range of temperature.

The experiment was undertaken to see whether the rates of carbon-dioxide assimilation of the leaves of a plant remain the same during the same months in a year. A series of experiments were repeated with the leaves of *R. communis* L. in July–August. If the rates of carbon-dioxide assimilation depended upon the seasonal activities of leaves the same rates of carbon-dioxide assimilation should be obtained for the same season. The results given below can be compared with those in Table VI.

TABLE VIII.  
*Ricinus communis* L.

Leaf No.	Temp. °C.	July to August.	Assimilation No. <i>W.</i>	December to April.	Assimilation No. <i>W.</i>	Leaf No.
		Assim. in one hour per sq. dm. leaf area.		Assim. in one hour per sq. dm. leaf area.		
		gram.		gram.		
63	25	0.0195	0.016	0.0088	0.0086	39
64	26	0.0178	0.017	0.0107	0.0098	40
65	27	0.0201	0.020	0.0129	0.011	41
66	28	0.0246	0.027	0.0143	0.014	42
67	29	0.0290	0.029	0.0159	0.015	43
68	30	0.0322	0.032	0.0177	0.016	44
69	31	0.0365	0.033	0.0181	0.017	45
70	32	0.0400	0.036	0.0225	0.019	46

On comparing the rates of assimilation given in Table VI for the leaves of *R. communis* L. with those given for the leaves of the same plant in the table above, it could be seen that in both of them the rates of assimilation and the assimilation numbers run very closely.

TABLE IX.  
*Ricinus communis* L.

Leaf No.	Temp. °C.	CO <sub>2</sub> assim. per sq. dm. leaf area per hour.	Chl. content per sq. dm. leaf area.	Water-content per sq. dm. leaf area.	Assimilation No. <i>CH.</i>	Assimilation No. <i>W.</i>
		gram.	gram.	gram.		
76	25	0.0112	0.00106	1.013	10.59	0.011
77	26	0.0118	0.00085	1.089	20.82	0.016
78	27	0.0206	0.00111	1.004	18.56	0.021
79	28	0.0304	0.00104	1.080	29.23	0.028
80	29	0.0252	0.000986	1.030	25.45	0.025
81	30	0.0347	0.00109	1.018	31.83	0.034
82	31	0.0378	0.00127	0.984	29.78	0.037
83	32	0.0424	0.00116	1.014	36.55	0.042
84	33	0.0507	0.00144	1.274	34.51	0.041
85	34	0.0493	0.00115	0.993	42.87	0.049
86	35	0.0563	0.00136	1.049	41.40	0.054
87	36	0.0405	—	1.021	—	0.040
88	37	0.0345	—	1.013	—	0.053

On examining the results obtained with the leaves of the three above-mentioned species it is noticed that the rate of photosynthesis is the highest in *H. annuus* L., and lowest in *A. asiaticum* G. Don., at all temperatures, while the rate of carbon-dioxide assimilation of the leaves of *R. communis* L. lies between the two. *H. annuus* L. has the maximum rate of carbon-dioxide assimilation at 36° C., and it is 106 mg. per square decimetre per hour; in *R. communis* L. it is 54 mg. at 36° C., and in *A. asiaticum* G. Don., 44 mg. at 34° C. On examining the water-contents of the leaves

in the three species, it is noticed that the amount of water per unit area is in the same order. In *Helianthus*, water-content of the leaves per unit area fluctuates between 1.6 grm. and 2.18 grm., in *R. communis* L. from 0.883 to 1.188 grm., and in *A. asiaticum* from 0.585 grm. to 0.744 grm. The ratios between the maximum rate of assimilation in the three species are 2:1:0.8, and the maximum water-contents of the three plants in the same order are in the ratios 2.1:1.1:0.7. Since the water-contents and the rates of carbon-dioxide assimilation are both of highest values in *H. annuus*, of medium values in *R. communis* L., and of low values in *A. asiaticum* G. Don., the assimilation numbers *W* run very closely at all temperatures

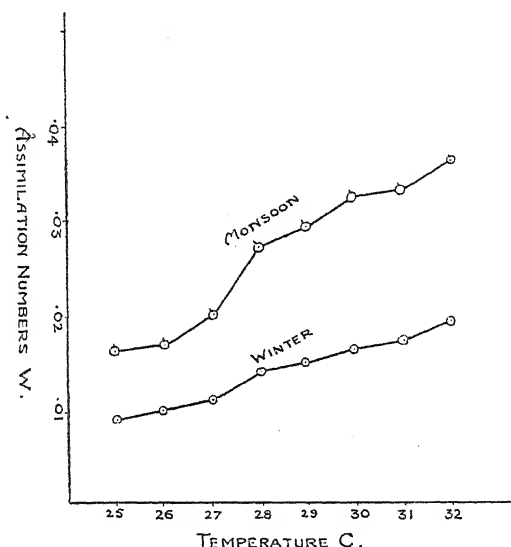


FIG. 6. Curves illustrating the differences in the seasonal photosynthetic activities of the leaves of *Ricinus communis* L. at different temperatures.

in *H. annuus* L. and *R. communis* L., but the assimilation numbers *W*, from 31° C., are higher in *A. asiaticum* G. Don. than those in *R. communis* L. and *H. annuus* at the same temperatures. This is probably due to the low water-content and comparatively higher value of the rate of assimilation in the former. In the other two cases the water-contents are relatively high and are in excess, while in *A. asiaticum* G. Don. it may be in relative minimum, so the assimilation numbers *W* are low in *R. communis* L. and *H. annuus*, and higher in *A. asiaticum* G. Don., as the water-contents are closely related to the rates of assimilation.

Since no such simple relation exists between the chlorophyll-content and the rate of carbon-dioxide assimilation, the assimilation numbers *CH* are variable and do not show any regular increase and decrease as the temperature is raised in all the three species. Similarly, on comparing the

assimilation number *CH* of the three species, they do not run closely, as Table XI below shows.

TABLE X.

Temp. °C.	<i>Helianthus.</i> Assim. No. <i>W.</i>	<i>Ricinus.</i> Assim. No. <i>W.</i>	<i>Abutilon.</i> Assim. No. <i>W.</i>
25	—	0·016	0·019
26	—	0·017	0·022
27	—	0·020	0·023
28	—	0·027	0·027
29	—	0·029	0·034
30	—	0·032	0·040
31	0·036	0·033	0·045
32	0·036	0·036	0·044
33	0·044	0·044	0·040
34	0·049	0·046	0·061
35	0·050	0·049	0·053
36	0·053	0·056	0·047
37	0·035	0·042	0·044

TABLE XI.

Temp. °C.	<i>Helianthus</i> <i>annuus</i> L.	<i>Ricinus</i> <i>communis</i> L.	<i>Abutilon</i> <i>asiaticum</i> G. Don.
Assimilation No. <i>CH.</i>			
25	—	12·6	15
26	—	39	15
27	—	23	16
28	—	25	14
29	—	47	10
30	—	45	29
31	74	32	74
32	93	32	91
33	92	46	67
34	102	42	56
35	94	41	80
36	96	41	45
37	61	52	103

From the above data it appears that the assimilatory activities of leaves in general show a closer relationship with water-content than with the chlorophyll-content.

On account of the higher rates of assimilation in the leaves of *R. communis* L. than those in the leaves of *A. asiaticum* G. Don., the maximum assimilatory point is at a higher temperature (36° C.) in the case of *R. communis* L. than in *A. asiaticum* G. Don. In order to test the point that the assimilatory rate of the leaves of *A. asiaticum* G. Don. falls after 34° C. (Table VII), experiments were repeated at 35° C., 36° C., 37° C. In all the sets the assimilatory rate fell, as the following results show (Table XII).

The same was the case with the leaves of *Ricinus communis* L. and *Helianthus annuus* when the rate of assimilation fell at 37 C. (Table XIII).

TABLE XII.

*Abutilon asiaticum* G. Don.

Leaf No.	Temp. °C.	CO <sub>2</sub> assim. per hour per sq. dm. leaf area.	Water-content per sq. dm. leaf area.	Assimilation No. W.
		gm.	gm.	
9	35	0.0177	0.637	0.028
10	36	0.0159	0.590	0.023
11	37	0.0142	0.610	0.023
12	35	0.0176	0.634	0.028
13	36	0.0136	0.678	0.020
14	37	0.0086	0.647	0.013
36	35	0.0182	0.6000	0.030
37	36	0.0132	0.5925	0.023
38	37	0.0078	0.6420	0.012

TABLE XIII.

*Helianthus annuus* L.

Temp. °C.	Assimilation No. W.	Assimilation No. W.
35	0.0935	0.0953
36	0.1067	0.1088
37	0.0728	0.0704

TABLE XIV.

Leaf No.	Temp. °C.	CO <sub>2</sub> assim. per sq. dm. leaf area.	Water-content per sq. dm. leaf area.	Assimilation No. W.
		gm.	gm.	
89	33°	0.0507	1.274	0.040
90	33°	0.0493	1.178	0.041
91	33°	0.0478	1.199	0.040

TABLE XV.

*Helianthus annuus*.

Leaf No.	Temp. °C.	CO <sub>2</sub> assim. per hour per sq. dm. leaf area.	Water-content per sq. dm. leaf area.	Assimilation No. W.
		gm.	gm.	
105	37	0.0728	2.016	0.035
107	37	0.0704	1.89	0.036
109	37	0.0629	1.80	0.035
108	35	0.1088	1.96	0.054
110	35	0.3953	2.80	0.053

*Phaseolus vulgaris*.

116	28	0.0941	1.625	0.058
121	28	0.0959	1.673	0.057

If the water-content of a leaf, as the results above show, is so closely related to its rate of carbon-dioxide assimilation, the assimilation number

*W* should remain constant when several readings are taken at any one temperature in a season. This point was put to test by making measurements of the rate of assimilation of the leaves of *Ricinus communis* L. at 33° C. in the last week of August 1929 (Table XIV).

Similar experiments were performed with the leaves of *H. annuus* L. and *Phaseolus vulgaris* (Table XV).

#### CONCLUSIONS.

The experimental evidence obtained leaves no room for doubt that water-content of a leaf is an important internal factor to which the photosynthetic activities of leaves is related. The method evolved by Dastur and Buhariwalla (5), has enabled us to show that the influence of the water-content in this important process is greater than that of the chlorophyll-content. Similarly, the chlorophyll-content, as shown by Dastur and Buhariwalla (5), is itself influenced to some extent by the water-content of the leaf. The role played by the water-content in photosynthesis is not wholly unexpected, as the importance of water to other life processes of a plant is very well known. But no one previous to Dastur had taken into account the water-content of a leaf when the relations of other external and internal factors with the photosynthetic processes were being investigated.

It is not possible to explain the way in which water plays such an important part in this process, but it may be suggested that the rate of diffusion of carbon dioxide within the assimilatory cells, and the removal of translocatory products may be affected by the water-content of the cell-walls and the cell-contents. The amount of water may also increase or decrease the velocity of reaction of the chemical stages of the process.

#### SUMMARY.

1. The continuous gas-current apparatus devised by Dastur (4) was used with some modifications to measure the rate of carbon-dioxide assimilation in this investigation.

2. The relationship between the *water-content* and the rate of assimilation of a leaf is expressed as assimilation number *W*.

The term assimilation number used by Willstatter and Stoll (17) to show the relationship between *chlorophyll-content* and the rate of carbon-dioxide assimilation is referred to here as assimilation number *CH*.

3. In the case of all the plants investigated, with the increase of temperature the assimilation numbers *W* rise up to a certain point and fall with further increase of temperature. The assimilation numbers *CH* do not show any such regularity.

4. The maximum rates of carbon-dioxide assimilation in *Abutilon*

*asiaticum* G. Don., *Ricinus communis* L., and *Helianthus annuus* L. are reached at 34° C., 36° C., and 36° C. respectively.

5. The rates of assimilation in *A. asiaticum* G. Don. and *R. communis*, L. show seasonal variability. The rates of assimilation of the two plants are higher in the monsoon months than in the winter months.

6. The values obtained for assimilatory rates are highest in *H. annuus* L., medium in *R. communis* L., and lowest in *A. asiaticum* G. Don.

The values for the water-contents in the three plants are in the same order.

7. The values of the assimilation number *W* show constancy in the plants investigated in a series of experiments at a fixed temperature, as should be the case if the assimilatory activity of a plant depended upon the water-content.

8. The results show that the CO<sub>2</sub> assimilation of leaves is more clearly related to the water-content than to the chlorophyll-content.

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# A Trisomic *Oenothera*.<sup>1</sup>

BY

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With Plate II and one Diagram in the Text.

## INTRODUCTION.

THE literature on the cytology of trisomic *Oenotheras* is not very extensive. Gates and Thomas (9) published a paper on the behaviour of the nucleus throughout meiosis in *Oc. mutant lata* and mutant *semilata*. Håkansson (10) considered some trisomic mutations of *Oe. Lamarckiana* including *Oe. pulla*, *curta*, *cana*, *stricta*, *lata*, *dependens*, *pallescens*, *liquida*, *longepetiolata*, but only figures stages of the heterotypic division, up to metaphase, in the pollen mother-cell.

Among the cultures at the Royal Botanic Gardens, Regents Park, in 1930, were plants grown from seed obtained from Professor de Vries in 1929. They were derived from self-fertilized plants of:

- (1) *Oe. Lamarckiana cana*, our culture No. 3/30, Row I, plants 1-5.
- (2) *Oe. Lamarckiana lata*, our culture No. 4/30, Row I, plants 1-7.
- (3) *Oe. Lamarckiana pallescens*, our culture No. 5/30, Row I, plants 1-2.

The aim of the work was to make a study of these plants with a view to elucidating the cytological behaviour of trisomic (= 15 chromosome) forms of *Oenothera*.

## DESCRIPTION OF CULTURES.

The season 1930 was very wet and cold, with the result that all the cultures were late in development, the plants mentioned above were particularly slow in growing. Reference to the culture book shows that none of these plants was a typical trisomic in appearance, with the exception of 3/30, I, 2, which was a questionable *cana*. It flowered earlier than the others (August 20), and had only 14 per cent. bad pollen. Most of the plants remained in the rosette stage.

Of the five plants in culture 3/30, by September 20, I, i, was in flower with the appearance of *rubrinervis*; I, 4, I, 5, had the vegetative characters

<sup>1</sup> Part of thesis approved for the Degree of Master of Science in the University of London.

of *rubrinervis* with very small flower stems and buds; I, 3, was a dwarf at the rosette stage, and remained so until October 10, when it had a short stem with buds which fell off, no flowers came to maturity, the pollen in these buds was 37 per cent. bad. I, 2, was self-fertilized, and crossed with *Oe. blandina* successfully.

Of the seven plants in culture 4/30, I, 2, from the very first was larger and different in appearance from the rest. The latter gave the appearance of *oblonga* at rosette stage. I, 2, was the only plant which came to maturity. It flowered by September 5, and was like a large *Lamarckiana*; it was provisionally labelled as *semilata*. The pollen was 35 per cent. bad, and the anthers were very thin. Throughout the season the rest of the plants remained in the rosette stage. I, 2, was self-fertilized, and crossed with *Oe. blandina* and other forms successfully.

Of the two plants in culture 5/30, I, 1, was branched from the base, had short, broad leaves, and red flower buds. It flowered by August 20. The flowers were the size of *Oe. biennis*, with pollen only 7 per cent. bad. The whole plant was more brittle than *rubricalyx*. Self-fertilization and crossing with *Oe. blandina* was very successful. I, 2 was a dwarf with three stems, having small leaves and compact terminal rosettes. It never had flower buds.

#### MATERIAL AND METHODS.

Material for cytological examination was very difficult to procure on account of the failure of the plants to form stems and buds. The method of procedure was similar to that described in Gates and Goodwin (8).

All the material from collections made during August and September was examined by means of wax sections, no smear preparations were made on account of the lack of material. Sections were cut at thicknesses varying from 9–11  $\mu$ , the majority at 10  $\mu$ . This was necessary owing to the poor physiological condition of the pollen mother-cells when fixed. Throughout the investigations slides were mordanted overnight in 2½ per cent. iron alum, washed for an hour the next morning, stained in ½ per cent. haematoxylin for three hours, washed, then de-stained with alcoholic picric acid solution, the percentage of acid varying with the stage to be de-stained. The slides were then rinsed in 70 per cent. alcohol, and kept for several hours in a solution of lithium carbonate in 70 per cent. alcohol in order to remove excess picric acid. I found the results were clearer in these difficult cases after the use of picric acid; the brown coloration from the use of iron alum in prolonged de-staining is avoided, as is the possible fading due to insufficient washing after the use of acid alcohol as a de-staining agent.

Preliminary examination of the epidermis of petal cells showed that only one plant from these cultures was a trisomic form (Pl. II, Fig. 1), 3/30 I, 4, the *cana* family. 3/30, I, 5, although similar in appearance, had

no contents to its anthers, and only showed fourteen chromosomes in the somatic cells. Further examination of the pollen mother-cells in 3/30, I, 2, which was considered a doubtful *cana*, showed a ring of six chromosomes and four free pairs at diakinesis (Pl. II, Fig. 13), placing it as a half-mutant. No *Oe. Lamarckiana* was found in this culture. The supposed *semilata* 4/30, I, 2, the only plant which was available in the culture for cytological examination, proved itself to have a ring of twelve chromosomes and a free pair, like *Oe. Lamarckiana*, at diakinesis. 5/30, I, i, from the *pallescens* culture was found to be a half-mutant with a ring of six and four free pairs of chromosomes at diakinesis. Hence the detailed cytological examination was carried out on 3/30, I, 4, a dwarf plant which formed buds very late in the season. It never flowered, so it was impossible to examine its pollen, and the cytological material failed to show enough anthers with pollen grains to calculate successfully the proportion of good to bad pollen, neither was it possible to self- or cross-fertilize the plant with any other form. Examination of the slides showed that no diakinesis stages were present. The majority of anthers contained pollen mother-cells showing stages from metaphase of the first division<sup>1</sup> to a few with the formation of tetrads at the close of the second division. A few anthers showed some mature pollen. Hence a study was made of:

- (a) the arrangement of the fifteen chromosomes at the first metaphase with special reference to the extra chromosome,
- (b) the distribution of the chromosomes after the first metaphase.

#### OCCURRENCE AND ORIGIN OF TRISOMIC OENOTHERAS WITH SPECIAL REFERENCE TO *Oe. CANA*.

*Oe. cana* is mutant from *Oe. Lamarckiana*. It was first noticed by de Vries from a plant in the third generation of a *lata* × *Lamarckiana* cross in 1906-7. The type is like *Lamarckiana*, but is characterized by narrow leaves, short blades, long petioles, grey coloration, long flower buds, spikes less dense than in *Lamarckiana* with cylindrical fruits bearing few seeds. Its chief distinguishing mark is that the four sepal tips are bent to one side.

The frequency of *cana* mutants from *Lamarckiana* is 0.03 per cent.; from *lata* up to 9 per cent. On selfing, *cana* gives *cana*, *Lamarckiana*, and some *Nanella*.

In crosses with other species or with *Lamarckiana* and its derivatives it follows the type of behaviour seen in *scintillans* and *lata*. The characters of *cana* are handed down through the ovules and not through the pollen in such crosses.

<sup>1</sup> In the description of the observations made the term *first division* is used in place of *heterotypic division* and *second division* in place of *homotypic division*.

*Pallescens* from derivatives of *Lamarckiana*, on selfing, gives, in addition to dimorphic progeny, *cana*, *liquida*, *scintillans*, *lata*, *albida*, and *rubrinervis* mutants up to 4 per cent.

*Lata* crossed with *Lamarckiana* gives rise to *cana*, *pallescens*, *lactuca*, and *liquida*.

The occurrence of *Lamarckiana*, half-mutants, and trisomics is to be expected in the progeny of the self-fertilized trisomic form.

Hence the explanation of the appearance of *Lamarckiana*, half-mutants and trisomic in the plants raised from seed, the result of self-fertilizing certain trisomic *Oenotheras*.

#### CYTOLOGICAL DESCRIPTION.

Arrangement of pollen mother-cells was in a single row throughout the anther when seen in longitudinal sections of flower buds; there appeared to be no difference in size of the pollen mother-cells compared with diploid forms.

The earliest stage available shows the end of diakinesis in a cut cell (Pl. II, Fig. 2), where a cut chain of nine chromosomes, the remainder at lower focus, and a free bivalent occur. I failed to find the tenth chromosome of the chain in either of the adjoining sections, probably because it was close to the membrane, and was pushed out by the knife in cutting.

There may have been thirteen chromosomes in the chain and a free bivalent. At the few earlier stages available two free bivalents and a chain or ring of eleven chromosomes were indicated. At the first metaphase there are several small chains; chains of five, seven, and three are common, together with ring-and-rod bivalents, and in about twenty per cent. of cases examined, a free chromosome—a univalent. The majority of cases show that the extra chromosome is attached either to the chain or, in all probability, as a member of a free pair of chromosomes. Various types of association occur, all of which are modifications of the arrangement shown in the diagram.

#### SOME POSSIBLE TYPES OF CHROMOSOME CONFIGURATION AT FIRST METAPHASE. (See Diagram for derivation.)

1. A ring of twelve chromosomes plus one bivalent, plus one univalent.
2. A chain of twelve chromosomes plus one univalent attached to chain, plus one free bivalent.
- \*3. A chain of thirteen chromosomes plus one bivalent.
4. A chain of eleven chromosomes plus one rod bivalent, plus one ring bivalent.
5. A chain of eleven chromosomes plus two ring bivalents.
- \*6. Chains of seven and five chromosomes plus one bivalent, plus one univalent.

\*7. A chain of ten chromosomes plus one ring-and-rod trivalent, plus one ring bivalent.

\*. Actually found, 4, 5 indicated in stages previous to diakinesis. A Y-shaped group of three chromosomes is shown in Pl. II, Fig. 5 *a*, *b*,

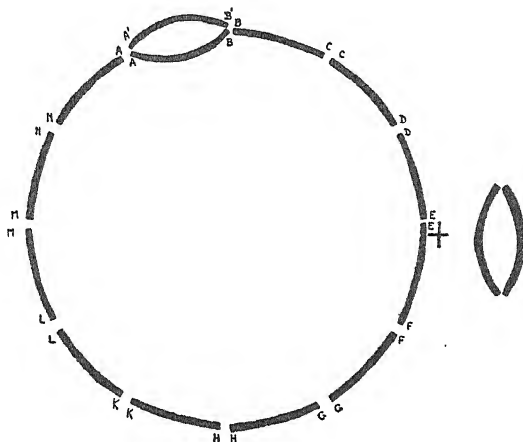


Diagram of ring and rod chromosomes with attachment of extra chromosome such as would be formed were all possible metaphase chiasmata established and maintained. The trisomic configurations are modifications of this.

and *i*; a ring-and-rod in Pl. II, Fig. 5 *e*, *f*, *f*, *g*; a branched chain in Pl. II, Fig. 6 *d*.

Pl. II, Figs. 5 *a*, *b*, *h*, shows stages in formation, by terminalization, of a triple chiasma. Pl. II, Fig. 6 *e*, shows an early anaphase with a rare kind of arrangement of chromosomes in which a pair of chromosomes has another chromosome (part of a chain of four) attached directly to it. It is one of the possible arrangements of the chromosomes in a trisomic and other heteroploids, following chiasma formation in small interstitial regions of two chromosomes otherwise non-homologous.

Pl. II, Fig. 6 *d*, is another example showing a branched chain of chromosomes with a large interstitial chiasma, *c*. The cell in which this occurs has been cut, so that the connexions of the rest of the chromosomes have been lost. The configuration shown is one in which four chromosomes have been concerned in the exchange of partners at an earlier stage in the meiotic history (at early prophase).

That chiasmata are more common in a trisomic than in diploids is shown by the many examples of incompletely terminalized chiasmata shown in Pl. II, Fig. 6 *a-e*, taken at random from metaphase and anaphase stages of the first division in the pollen mother-cell nuclei of the trisomic form.

In Pl. II, Fig. 6 *a*, the middle pair of chromosomes shows a large chiasma in a rod bivalent, the extra chromosome being present as a

univalent; Fig. 6 *b* has an interstitial chiasma at *c* in the chain, which looks almost as large as a chromosome. Similar interstitial chiasmata occur in the chain of five chromosomes in Fig. 6 *c*, which also shows a univalent. (The position of the univalent may be the result of the microtome knife), Fig. 6 *e*, shows a chiasma in a rod bivalent at *c*.

These configurations agree with some of those found by Catcheside (1) in triploid *Oe. pycnocarpa*. Reference to his text (Fig. 1) shows that I have found figures similar to *a, b, c, e, f, g*, and *k*, but not *h, l*, or *m* in my trisomic material (neither *l* nor *m* is possible in a trisomic, since there is only *one* extra chromosome).

#### BEHAVIOUR OF CHROMOSOMES AT LATE ANAPHASE—INTERKINESIS AND THE SECOND DIVISION.

After metaphase the chromosomes prepare to pass to their respective poles. In the majority of cases a 7-8 distribution occurs, the extra chromosomes having passed to the pole with one of the two groups of seven chromosomes; at the second division there is a regular formation of tetrads containing 7-7 and 8-8, chromosomes, giving rise to four pollen grains containing, presumably, viable gametes. Those on meeting gametes from normal diploids or those of other trisomics should give rise to normal diploids and more trisomic forms. This behaviour is similar to that observed by other workers, e.g. de Vries, Gates, Håkansson. All these workers have shown, however, that eggs containing eight chromosomes appear to be viable, while pollen with eight chromosomes is rarely viable. This lack of viability may be due to some unknown physiological causes.

In other cases, however, irregularity of behaviour is observed, due to:

(1) the presence of incompletely terminalized chiasmata and the consequent failure of chromosomes to reach the poles in time to be included in the interkinetic nuclei;

(2) irregular behaviour of the univalent.

In Pl. II, Figs. 5 *f* and 6 *a-e*, where examples of chromosome arrangement at metaphase and anaphase of the first division are shown, it will be seen that separation of the members of a bivalent and of adjacent chromosomes of a chain where chiasmata occur, is liable to cause those chromosomes to be 'held-up' on their passage to the poles, and it was often noticed that a pair of chromosomes was left outside, stranded between the two nuclei in interkinesis, unable to pull away from each other in time to be included. There is a mechanical hindrance (Pl. II, Fig. 7).

It has been shown by Gates (6, 7) in trisomic mutants, as well as in triploid *Oenotheras*, that lagging of chromosomes occurs at heterotypic anaphase. In a triploid *Oenothera* hybrid as many as six chromosomes were left out at this stage.

Perhaps the members of the pair may be included later, e.g. Pl. II, Fig. II, where one chromosome has just been included, and the other is about to be. In other cases the lagging bivalent encloses itself in its own nuclear membrane, while the rest of the chromosomes have entered the interkinesis nuclei (Pl. II, Fig. 10).

With regard to the univalent when unattached it may:

- (1) pass at random to either pole at anaphase of the first division,
- (2) divide,
- (3) be left out of the nuclei in interkinesis,
  - (a) then come into the range of the spindle forces at the second division,
  - (b) fail to be included, even in the nuclei of the second division.

As a result of irregularities, both at the first and second homotypic divisions, diverse forms of gametes are obtained with varying contents, some of which will prove to be viable, others will not. They may contain 6, 7, or 8, or occasionally 9 chromosomes, sometimes  $5\frac{1}{2}$ ,  $6\frac{1}{2}$ ,  $7\frac{1}{2}$ , or  $8\frac{1}{2}$  chromosomes.

Gates (6, 7) showed that in trisomic mutants of *Oe. rubricalyx* × *Hewetti*, unlike the trisomic *lata* which it resembled, the pollen was viable, and he also found pollen grains containing eight chromosomes which were functional. This is unusual, for, as a rule, such pollen grains are non-functional.

Pl. II, Fig. 9, shows an interesting case where there is great irregularity of behaviour. Here on one spindle is a 7-7 distribution with a half chromosome *L* stranded on the spindle (one of the seven on the left-hand side is pushed out). The other spindle shows a 5-5 distribution with another lagging half chromosome *L* in the same position as on the 7-7 spindle. In the middle of the cell at top focus lies another spindle which shows two bodies at either end. Unfortunately, the cell was just sliced at  $\alpha$ , and the pieces lost in the making of the preparation. The interpretation apparently is that a pair of chromosomes, probably as the result of a chiasma, failed to be included in the daughter nuclei. In addition a univalent divided, one half passing to each pole.

At the second division the seven chromosomes split, and the split halves passed to their respective poles, but the split half of the univalent lagged on the spindle as it had no further splitting to do, but could pass at random to either pole. Similarly, the five chromosomes in the other nucleus split at the second division, and proceeded to their respective poles, and the half-chromosome from the univalent was left on the spindle where it might pass at random to either pole.

The spindle in the middle of Pl. II, Fig. 9, supports a pair of chromosomes which had been left out at the first division; they then

formed a spindle as in a normal first division, and the stage represented in the nucleus figured shows the members of the pair which have separated, and in the uncut end the separated chromosome has split into two halves, preparatory to the formation of the second spindle; the cut ends of the other member of the original pair lie at the bottom end of the spindle *a*. Hence there is a  $7\frac{1}{2} + 5\frac{1}{2}$  distribution at the second division, together with the pair of chromosomes at the end of the first division. This would lead to the formation of gametes with varying chromosome numbers depending on whether the half chromosomes on each spindle managed to get included in one or other of the daughter nuclei before tetrad formation was completed. In any case half the gametes would be non-viable, as they would have less than the necessary complement of seven chromosomes each.

Irregular pollen development is to be expected in trisomics including diads, triads, and hexads, in addition to the normal tetrad. I examined what little material there was at this stage, but did not find good examples of diad or triad formation; an example of hexad formation is shown in Pl. II, Fig. 12. The large size of the two medium-sized nuclei suggests that these extra nuclei contain more than a half-chromosome each. In all probability only two of the nuclei will give viable gametes, perhaps none.

Pl. II, Fig. 9 *a*, is similar to Fig. 9 in some respects. Evidently there was an 8-7 separation of chromosomes on the first spindle. The second spindle in polar view shows a slight lag in one half-chromosome (*d*). On the other second spindle five chromosomes have separated while the other two are lagging, half of one having been displaced by the knife.

#### EXPLANATION AND DISCUSSION.

As was pointed out earlier in this account, the greater part of the irregular nuclear behaviour in this trisomic may be regarded as dependent upon the presence of chiasmata, that is to say, at a very early prophase there must have been exchange of partners between chromosomes.

This is shown where two chromosomes have been concerned in an exchange of partners (Pl. II, Fig. 6 *d*) with a large interstitial chiasma at *c*. It is again shown by the presence of the Y-shaped trivalent grouping with a triple chiasma, either alone or at the end of a chain in the ring-and-rod formation, and in the less frequently occurring branched chain of a quadrivalent where there has been exchange of partners between three chromosomes (Pl. II, Fig. 5 *a*, *b*, *i* and 6 *e*).

Often in these compound chiasmata, one of the chiasmata is by no means fully terminalized, and appears as an irregular lump, the *Querarm* of Håkansson. Sometimes it is almost as large as a chromosome, and in



the earlier paper of Gates and Thomas (9) two interstitial chiasmata were actually figured as chromosomes in a drawing of a cut nucleus at heterotypic metaphase ((9), Pl. XXXVI, Fig. 37 *a*), where two adjacent pairs of chromosomes have broken away from the rest of the chain, and are endeavouring to pass to their respective poles.

This brings into view another point, which has a great bearing on the subsequent behaviour of the chromosomes. After the crossing-over between the constituent chromatids of the longitudinally split chromosomes has taken place, previous to diakinesis, there is a tendency for the chiasmata so formed to move to the ends of the segments of the chromosomes, and this process has been termed 'the terminalization of the chiasmata'. If the chiasma is interstitial or sub-terminal, then the tendency will be for movement to occur to make the terminalization complete; at the same time the chromosomes are coming under the influence of the spindle forces, and are being pulled apart at early anaphase towards their respective poles. In a nucleus where all the chiasmata are nearly or completely terminalized at the time of disjunction there will be little or no mechanical hindrance and a normal 7-8 separation results with no lagging (Pl. II, Fig. 8). But if a large interstitial chiasma is present at this time, then there is considerable mechanical hindrance due to the movement of the chiasma in order to complete terminalization together with the pulling force of the spindle. Consequently the other chromosomes without such hindrance at this time are able to travel to the poles, and form daughter nuclei at interkinesis, while the chromosomes, with incompletely terminalized chiasmata, are delayed near the region of the plate until terminalization has reached such a stage that the spindle forces are able to cause separation between the chromosomes. In this way chromosomes may be left out of the interkinesis nuclei altogether or just manage to be included. Hence the various irregularities which have been observed in the trisomic in question (Pl. II, Figs. 7, 9-19).

The other irregularity which occurs is due to the univalent which may pass at random to either pole at the first division or fail to do so, and then divide at the time of the second division, there coming into the range of attraction of the spindle forces; the half-chromosomes may pass at random to either pole there. This explains the lagging seen on spindles observed at the time of the second division. Occasionally more than one half-chromosome may be seen lagging on the spindle at this stage; this may be due to the postponed division of the univalent at the first division when it was enclosed in one of the daughter nuclei.

In the trisomic, observations show that there is a very frequent occurrence of interstitial chiasmata, and this may be explained as due to the extra chromosome, which is completely homologous with one other of the twelve ring-forming chromosomes of *Lamarckiana* and with the ends of

two other chromosomes, having paired up with short interstitial segments in other chromosomes with which portions of it are homologous (Pl. II, Fig. 6, and Diagram, p. 93).

The observations made on this trisomic *Oenothera* give further support to the view that *Oenothera* is parasynaptic (8). In order to explain the presence of chiasmata in the absence of direct observation of well-fixed early prophase stages in the trisomic form (although actually seen in a diploid *Oe. purpurata*), one has to presume that there has been pairing throughout the early prophase stages, with an attendant crossing-over, the formation of chiasmata and their consequent terminalization, which may, or may not, have been completed at the first division, owing to the mechanical hindrance they incur.

This also demonstrates the fact that it is to the advantage of an organism, sexually reproduced, that the chiasmata shall be completely terminalized in order that the daughter nuclei receive the normal quota of chromosomes, and that the maximum number of gametes formed, as a result of meiosis, shall be viable.

In the meiosis of a trisomic form, provided that there has been no mechanical disturbance that was not overcome by the time the chromosomes formed the interkinesis daughter nuclei, and that the univalent was included and passed to the poles at the second division in a normal way, then the pollen formed should contain gametes with 7-7, 8-8 chromosomes, and all four gametes should prove viable. (This is true of eggs with eight chromosomes, but rarely so of the pollen with eight chromosomes, however.) But with the exclusion of chromosomes at the first division (as a result of the mechanical hindrance of incompletely terminalized chiasmata) and their failure to enter the daughter nuclei at the second division, abnormalities in the chromosome content of the resulting gametes means that 50 per cent. or more, of the gametes will prove non-viable, and this would be a considerable drawback to the perpetuation of the form concerned. This is a possible cause of the low percentage of trisomics in progeny of selfed trisomic forms of *Oenothera*.

#### SUMMARY.

1. A description of cultures of plants from the self-pollination of certain trisomic *Oenotheras* is given.
2. The cytology of one trisomic form derived from *Oe. Lamarckiana cana* is described in detail, with special reference to the distribution of the chromosomes during meiosis.
3. Types of chromosome configuration at first metaphase are described. These include:
  - (i) a chain of thirteen and a ring bivalent ;

- (ii) chains of seven and five chromosomes, together with a ring bivalent and a univalent ;
- (iii) a chain of ten chromosomes with a ring-and-rod bivalent, together with a ring bivalent ;
- (iv) branched chains of four and five chromosomes.

4. Various examples of chiasmata are described and figured, including interstitial chiasmata between homologous ends of two and of three chromosomes.

5. The mechanical hindrance of incompletely terminalized chiasmata at the metaphase of the first division is regarded as the cause of the delayed separation of a pair of chromosomes.

6. Support is given to the view that *Oenothera* is parasynaptic, that crossing-over occurs, that terminalization of the chiasmata so formed must take place in order that viable gametes may be produced.

I carried out this work while research assistant to Prof. R. R. Gates, to whom my thanks are due, and I am also greatly indebted to Mr. Catcheside for his helpful criticism during the progress of the investigation.

DEPARTMENT OF BOTANY,  
KING'S COLLEGE, LONDON.  
July, 1931.

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## EXPLANATION OF PLATE II.

Illustrating Miss K. M. Goodwin's paper on 'A Trisomic *Oenothera*'.

All figures were drawn at table level with the aid of a camera lucida. A 2 mm. Zeiss apochromatic objective N.A. 1.4 and Zeiss oc.  $\times 20$  were employed for Figs. 1-6 and Fig. 13. Magnification  $\times 2,950$ . Figs. 7-12 using same lens  $\times 15$  oc. Magnification  $\times 2,000$ . Preparations fixed in Allen's Bouin. (Kihara's method). All figures are of trisomic *Oenothera cana* except Fig. 13 which is of a half-mutant from the same culture (3/30).

Fig. 1. Somatic metaphase showing fifteen chromosomes in trisomic *Oenothera*.

Fig. 2. Diakinesis in a cut cell showing cut chain and one free bivalent. (There were originally thirteen chromosomes in the chain.)

Fig. 3. Anaphase showing fifteen chromosomes, univalent at top, chain with triple chiasma and three free pairs of chromosomes at first division.

Fig. 4. Anaphase showing seven free bivalents and univalent chromosome at first division.

Fig. 5. *a-j* show various configurations involving triple and interstitial chiasmata (taken from metaphase and early anaphase stages of the first division). *a, b, i* show Y-shaped trivalent, *a*, and *b* show markedly interstitial chiasmata. *c, d, h* show quadrivalents with triple chiasmata. *e, f, g* show ring and rod trivalents. *j* shows a chain of three chromosomes with one interstitial and one terminal chiasma.

Fig. 6. *a-e* shows further examples of chiasmata taken from metaphase and early anaphase stages of the first division. *a*. Analysis of whole plate showing large interstitial chiasma *c* in rod bivalent; note univalent and quadrivalent. *b*. Chain of five chromosomes with large interstitial chiasma *c*. *c*. Analysis of whole plate showing two chiasmata in chain of five chromosomes, a univalent, a rod bivalent, a chain of five chromosomes with terminalized chiasmata. *d*. Branched chain of five chromosomes showing terminalized chiasma to left, large interstitial chiasma in middle at *c*, a univalent in lower focus. (Cell cut at top focus). *e*. Whole plate showing rare arrangement of chromosomes; abnormal connexion between chromosomes of chain and one chromosome of a ring bivalent. Note rod bivalent with chiasma *c*.

Fig. 7. Late anaphase, showing 7-7 distribution and lagging of one chromosome already split. Note late pulling apart of one pair of chromosomes due to a chiasma. One chromosome at bottom has already split.

Fig. 8. Oblique view of telophase at first division, showing 8-7 distribution.

Fig. 9. Telophase of first division, showing 7-7 distribution and 5-5 distribution with lagging half-chromosome (= one chromatid) on each spindle. Note spindle in middle at top focus with one pair of chromosomes from the first division excluded; they are about to start their second division independently. (Cell is cut at *a*.)

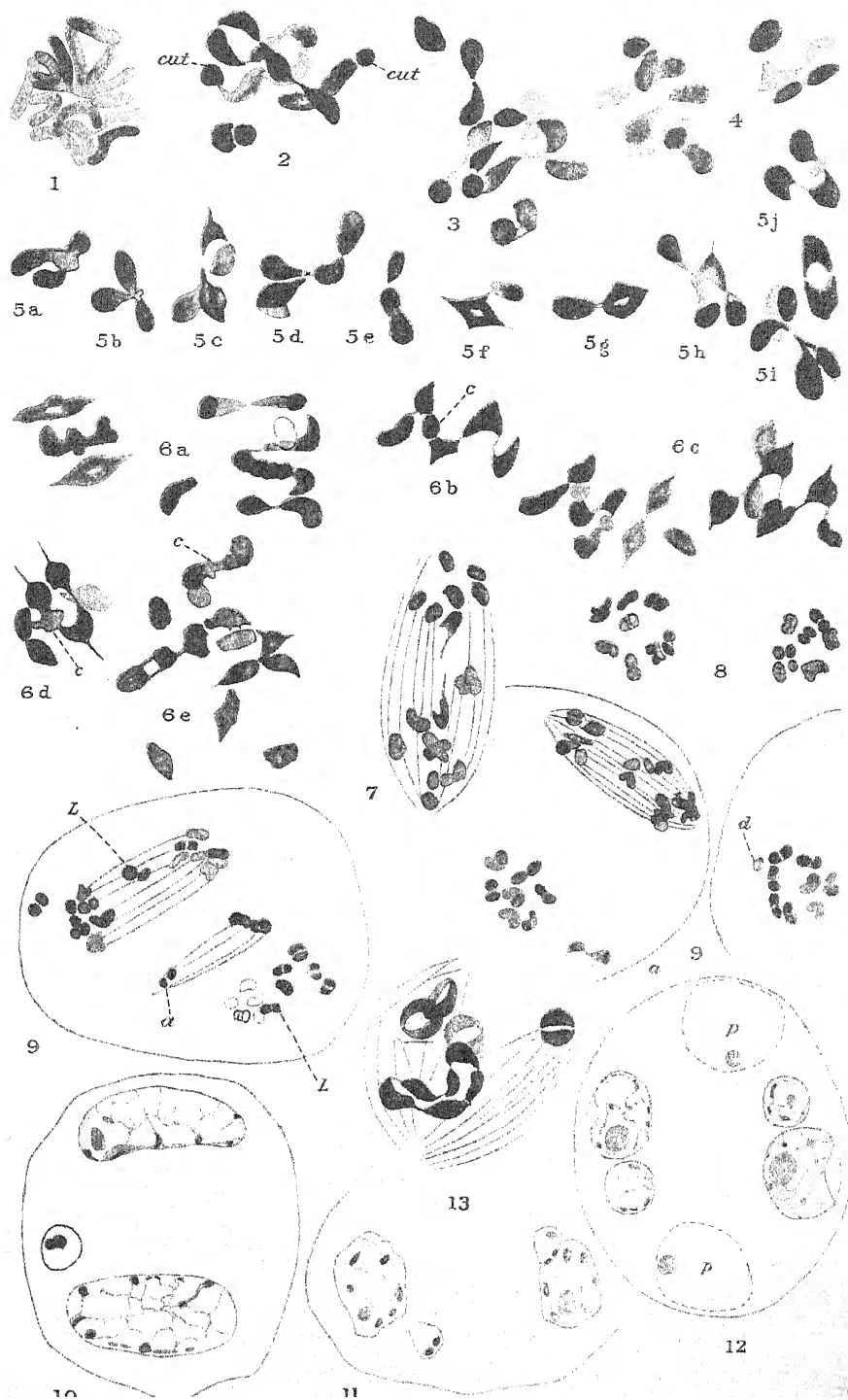
Fig. 9a. Analysis of metaphase of second division showing lagging of more than one half-chromosome on one spindle. Note 8-8 distribution in polar view with one lagging chromosome. *a*. Note half-chromosome pushed out into cytoplasm.

Fig. 10. Interkinesis showing independent nucleus formed by lagging univalent.

Fig. 11. Interkinesis, inclusion of one chromosome of a lagging pair, and near inclusion of other member of original pair.

Fig. 12. Hexad formation.

Fig. 13. Multipolar spindle in half-mutant. From same family as trisomic *Oenothera* shows ring of six, two interlocked ring bivalents, two free ring bivalents.





# Some Observations on *Bifurcaria tuberculata* Stackh.

BY

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(*Westfield College, University of London.*)

With seven Figures in the Text.

## I. INTRODUCTION.

LITTLE is known in detail of the morphology, anatomy, and reproduction of the Phaeophyceean genus *Bifurcaria* beyond brief descriptions and figures given by Kützinger (7 and 8) under the generic name of *Pycnophycus*, and by Thuret and Bornet (22). More recently some investigations on the method of branching were carried out by Grüber (5), and on the development of the conceptacle by Nienburg (14). An attempt is made in this paper to throw some light on the morphology and anatomy of the only British species, *B. tuberculata* Stackh. The other two species, *B. levigata* Kütz. and *B. brassicaeformis* Kütz., are confined to African coasts, together with Kützinger's variety of *B. tuberculata*—*sisymbrioides*.

The distribution of *B. tuberculata* is somewhat limited. It occurs abundantly on the coasts of Devon and Cornwall, on the south-west coast of Ireland (with its northern limit in the Roundstone neighbourhood of Co. Galway (3)), and in the Channel Islands. This species has also been found at Whitesands Bay, Pembrokeshire, Isle of Wight (Niton), and Portland. It also grows on the Atlantic coasts of France and Spain, and on West African shores as far south as Cape Agulhas (according to Pappe, Schousboe, and Grunow).

The material used for this investigation was collected in July, 1924, on the coast of Brittany between Cape Finisterre and St. Malo, and in April, 1926, on the Cornish coast between the Lizard and Land's End, and supplemented by material collected by Miss Westbrook, M.Sc., at Plymouth (Wembury) in July, 1926.

Usually the material was fixed immediately on the shore in the customary fixatives, the best of which was found to be Allen's modification of Bouin's fluid (11). An attempt was made to obtain mitotic figures by

<sup>1</sup> Mrs. F. M. Laing.

leaving portions of fronds in sea-water (with frequent changes) until such a time as they would normally be covered by the tide for an hour. As this procedure failed to produce the desired results, only limited cytological observations have been possible.

A short account of this species was read at the meeting of the British Association in 1927, and the paper accepted as a thesis for the M.Sc. degree in the same year, but publication has been hitherto deferred.

## II. MORPHOLOGY AND HABITAT.

*B. tuberculata* is seldom left uncovered at ebb-tide, but is found inhabiting rock pools, usually the deeper, narrower variety which form long creeks lying anywhere between mid-tide and low-tide level; this was especially noticeable on the Cornish coast, where *Bifurcaria* pools lay exposed for as much as fifty yards back from the water's edge, even at neap-tides.

The rocks in such regions are characteristically bare of covering algae, and are frequently barnacle-covered; the absence of species of *Fucus* is particularly striking. The pool associations, as observed on the Cornish coast, are poor in algal species, *Corallina* and *Cladophora* sp. being the most common. On the south coast of Devon and in Brittany, *Cystoscira ericoides* L. and *C. granulata* L. were frequently found associated with *Bifurcaria*, but this was not the case in Cornwall. At Wembury, near Plymouth, *Halidrys* is often present. It would seem that where *Bifurcaria* occurs it flourishes in such profusion as to crowd out almost all other genera.

Pools inhabited by *B. tuberculata* often present a characteristic appearance, the extreme tips of the fertile fronds being exposed above the surface of the water (Fig. 1); this is more obvious in the shallower pools, where the fronds grow upwards from the bottom. In narrower creeks the plant is attached to the sides and grows obliquely inwards and upwards to the surface.<sup>1</sup> This appearance was found to be so striking that the presence of *Bifurcaria* in pools could be detected from overhanging cliffs with a reasonable degree of certainty.

The morphology of the genus in question is interesting in view of the fact that it is one of the very few rhizome-forming members of the Fucaceae. The plant consists of an irregular, horizontal, rhizome-like structure bearing small, rounded protuberances and cylindrical, erect fronds; the rhizome is attached to the substratum by small and often insignificant adhesive discs. As the rhizome is easily pulled away intact from the substratum, the attaching discs appear to be peculiarly ineffective when compared with

<sup>1</sup> From information kindly supplied by Dr. Delf, it appears that the habitat and exposed tips are the same for *B. brassicaeformis* in S. Africa.



the extremely tenacious holdfasts of the majority of the Fucaceae. In spite of this, *Bifurcaria* plants are only very rarely found cast up on the shore in contrast with those of *Fucus*. This may be accounted for by the rela-

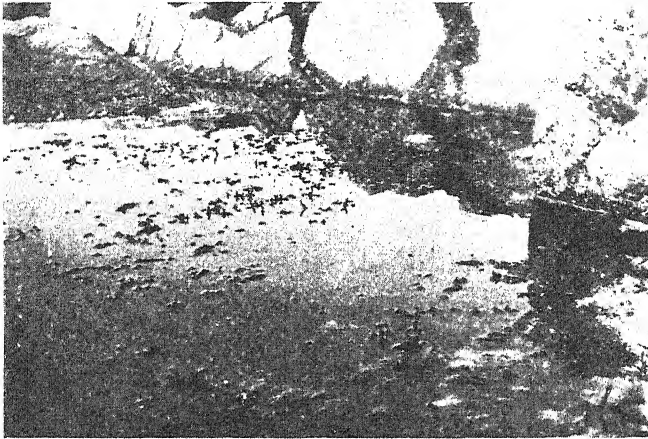


FIG. 1. Photograph of a *Bifurcaria* pool at Trestignel, Brittany, showing the exposed tips of the erect fronds.

tively sheltered position of the former, and possibly its radially constructed shoots offer less resistance to the action of the waves than do the flat fronds of *Fucus*.

The branching of the shoot system in *B. tuberculata* is made irregular in appearance on account of the random development of erect, horizontal, or dormant shoots. According to Grüber (5), the branching here is comparable with that of a monopodial *Halidrys* which has become horizontal, some of the branches developing into erect shoots, some growing horizontally, and others remaining dormant and represented by the characteristic rounded protuberances on the rhizome (see Fig. 5).

Old fertile fronds and young spring fronds may both occur in a single plant, the latter being a light olive brown and the former a very dark brown, almost black. In dried specimens this colour difference is not very obvious, but the young spring fronds are still recognized by the incurved ends of their ultimate branches (Fig. 2). The older fertile fronds may reach a length of some 12 to 18 inches, with a diameter, when fresh, of 2 to 4 mm. They are much branched, giving the plant a tufted bushy appearance. The fronds are not of uniform diameter throughout their length, but are constricted where they branch and at their point of origin from the rhizome. Air vesicles have been described for this species, and are figured by Kützinger (8) as occurring at the base of the old receptacles. Specimens from different localities vary quite definitely in the production of vesicles ;

about 100 Cornish and French specimens were examined, but only on rare occasions were vesicles observed,<sup>1</sup> whilst in material collected at Wembury, Plymouth, they were quite common. Mackay (12) has noted the abun-



FIG. 2. *B. tuberculata* Stackh. Habit drawing of a plant, showing fertile fronds (f.), young spring fronds (s.), and rhizome (r.). About  $\frac{1}{2}$  natural size.

dance of vesicles in material collected on the Irish coast; as no Irish material was available for this investigation, I have had no opportunity of confirming this observation.

By cutting young tips longitudinally, the lysigenous origin of the young vesicles is apparent (Fig. 3 c).

All the tips of the erect fronds eventually become fertile; these later die down leaving only their bases still attached to the rhizome.

The cylindrical receptacles vary in length from one-tenth to two inches; in these are embedded the conceptacles which are typically Fucaceous in form, and are irregularly arranged round the cylindrical receptacle. In the very young fertile tip, conceptacles are only apparent if the plant be held up against the light, when they are recognizable as minute dark spots on a

<sup>1</sup> I am indebted to M. Roger Meslin for a loan of herbarium specimens of this genus from the Institut Botanique, Caen.

light brown background. In older receptacles, on the other hand, the conceptacles are rendered conspicuous by a slightly tuberculate appearance of the originally smooth surface. This appearance, which is particularly

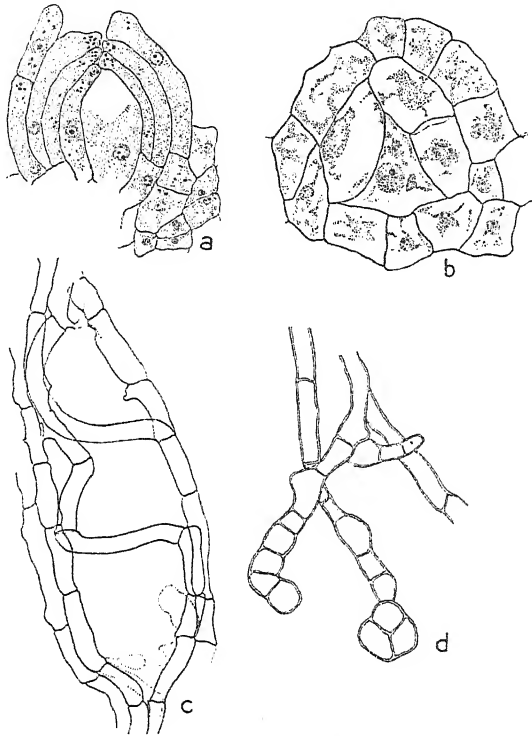


FIG. 3. *B. tuberculata*. (a) V.S. through apical cell of young fertile frond.  $\times 530$ . (b) X.S. apical cell.  $\times 580$ . (c) V.S. through young vesicle at tip of erect shoot, showing lysisogenous origin.  $\times 440$ . (d) Hyphae teased out from an old attaching disc, showing segmentation at the tips.  $\times 700$ .

noticeable in pressed material, accounts for the specific name of the plant. Old receptacles are capable of proliferating, becoming drawn out into new tips which produce new conceptacles. Little is known as to the periodicity and time of fruiting; material collected both in April and July was fertile, but the July specimens showed a greater proportion of young fertile tips and old proliferating ones.

The 'cryptostomata' or 'vegetative conceptacles' characteristic of so many genera of this group are absent in *B. tuberculata*.

Growth of the whole shoot takes place by means of a three-sided apical cell, as is typically the case in the Cystoseiro-Sargasseae. This cell is situated at the base of a mucilage-filled groove located at the apices of the branches and of the basal protuberances. The groove in a young fertile tip attains to a depth of about one-third mm. from the apex of the shoot. In the rhizome, the groove often appears to be V-shaped, due to the division of

the original apical cell with subsequent formation of three such cells. This triad arrangement of the apical cells is frequent in the rhizome, and was observed and mentioned by Grüber.

In cross-section, the apical cell was found to be invariably and distinctly triangular, and in longitudinal section the biconvex lens appearance is equally persistent (Fig. 3 *a* and *b*). This suggests that the apical cell, if isolated, might be compared in form to an object such as a Brazil nut standing up on end and having three equal sides. This, if cut in any plane except longitudinally (when the section is lens-shaped), would always appear triangular.

The segmentation of this cell takes place in the usual way, hour-glass segments being cut off on each side, these dividing first longitudinally and later transversely.

### III. ANATOMICAL STRUCTURE.

Wille's classification of the tissue-systems of the brown algae (24) is the most applicable in the case of *B. tuberculata*, and will be followed here.

#### (a) *Assimilating system.*

In *Bifurcaria* this consists of a single layer of small, radially elongated cells about half as broad as long (Fig. 4 *a*). The lateral and inner walls are thin, and in surface view the radial walls assume a wavy outline. The external layers of the outer convex walls appear to become converted into a mucilage-like substance forming a continuous layer over the surface of the assimilating cells. This covering has been called the 'cuticle', but it does not contain cutin. A modification of the outer cell walls is especially conspicuous within the apical grooves, where safranin and methylene blue both give their characteristic staining reactions for mucilage.

Lying along the radial walls of the assimilating cells are abundant plastids; in a cross-section of a young erect frond these are long and narrow and probably disc-shaped.

An increase in the number of cells in the assimilating layer is brought about by divisions perpendicular to the surface of the plant, whilst divisions parallel to the surface result in the formation, towards the interior, of cells which eventually form part of the storage system.

There is, obviously, no definite delimitation between the latter and the assimilating system, or between the storage and the conducting systems. It is often difficult to judge the exact extent of the assimilating tissue, as the periclinal divisions of the cells of the latter produce, towards the interior, small cells usually containing abundant phaeoplasts.

#### (b) *Storage system.*

Immediately below the superficial assimilating system lies an indefinite zone of 8 to 10 or more layers in depth (Fig. 4 *a*). The cells are from 4 to 6

times the diameter of the cells of the other two systems, and are very sparsely provided with plastids except in the outer two cell layers, which are presumably capable of assuming an assimilatory function.

In fresh material, the outer storage layers gave an intense red coloration if treated with vanillin and concentrated hydrochloric acid, thus indi-

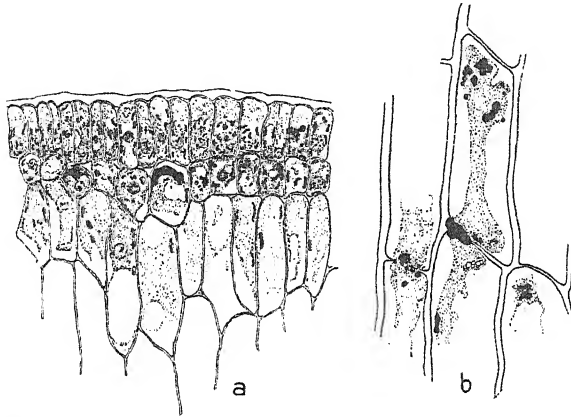


FIG. 4. *B. tuberculata*. (a) X.S. Young fertile frond near the tip, showing the 'assimilating' and 'storage' systems.  $\times 500$ . (b) Part of V.S. through the conducting system, showing oblique walls and lateral contraction of the cytoplasm.  $\times 350$ .

cating the presence of fucosan. Its presence in these cells would be expected if the fucosan were in reality a product of assimilation and not, as Kylin maintains, merely a by-product of this process.

Towards the interior of the frond, the storage cells gradually become elongated along the axis, and grade imperceptibly into the central conducting system. This elongation is probably due to successive inhibition of cell division in the more deep-seated cells, with the result that these become passively stretched, and their length may then attain to as much as six or seven times their breadth.

I have not been able to observe communicating pores in this or in the assimilating system, although such were easily distinguished in the central cells.

#### (c) *Conducting system.*

Running centrally through the length of the erect fronds, but not distinguishable in the rhizome, is a strand of narrow elongated cells somewhat comparable with the elements of the conducting strands of mosses. Near the tip of a fertile young shoot these cells are from three to six times as long as broad, and in older shoots four to six times as long as broad.

In longitudinal section it is seen that the transverse walls are frequently obliquely placed (Fig. 4 *b*), and radial communication between the cells is

established by means of pores. The tendency of the cytoplasm to contract laterally rather than longitudinally (Fig. 4 *b*) is strongly suggestive of protoplasmic connexions across the transverse walls, although such were not actually observed.

In *B. tuberculata*, as opposed to another species of the same genus—*B. levigata*, no 'Verstärkungshyphen' such as are characteristic of the Fuceae occur, except during the formation of the attaching disc.

The delimitation of the tissues in the rhizome is even more difficult to decide than in the erect shoot. The cells of the outer two or three layers vary only slightly in size, but the internal cells become progressively larger; this increases the difficulty of designating any particular layer or layers as definitely 'assimilating'. In the rhizome the outermost layer is not as laterally coherent as in the erect shoots, a certain amount of mucilage being present.

All cells of the rhizome are full of fucosan which is apparent as large spherical 'granules' due to fixation of the original vesicles.

Hyphal formation occurs only on the initiation of attaching discs, the hyphae arising from any of the cells internal to the two or three outer layers. The older discs are conspicuous as irregular protuberances on the under surfaces of the horizontal rhizome and attain in some cases to a considerable size (Fig. 5). The young discs are difficult to detect; it was found, however, that by leaving material in 50 per cent. alcohol for some days the small discs were rendered conspicuous on shrinkage of the intervening tissues and were now found to stand out as small whitish lumps, the youngest ones being located immediately below the advancing tips of the rhizome.

Of the other two species of the genus, *B. brassicaeformis* has a well-developed rhizome comparable with that of *B. tuberculata* but more massive, but *B. levigata* possesses a holdfast similar to that of *Fucus vesiculosus* in place of a rhizome. The attaching discs in *B. brassicaeformis* have a more regular lineal arrangement and a more nearly disc-like form than in *B. tuberculata*.

In their internal structure the discs are comparable with other holdfasts of the Fucaceae (Oltmanns (16)). A young disc is composed exclusively of hyphae which arise from the more central cells of the rhizome, grow obliquely downwards and become thickly intertwined to form a compact tissue. These hyphae are sparsely provided with transverse walls, but, where the tips grow out to the edge of the disc, cells are produced by segmentation backwards from the apex (Fig. 3 *d*), forming a pseudoparenchymatous tissue at the disc edge. Older discs are further strengthened by the formation of branch hyphae which pursue a course perpendicular to that of the original ones, and are conspicuous as short strands of several parallel hyphae running out to the disc margin, segmenting at the tips.

Such secondary hyphae have also been observed in the holdfasts of other members of the Fucaceae and are particularly striking in the holdfasts of *Halidrys siliquosa* (L) Lyngb.

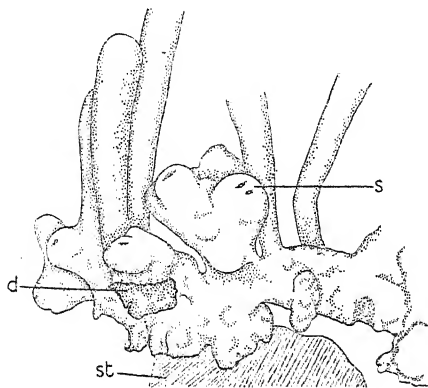


FIG. 5. Part of the rhizome of *B. tuberculata*, showing several attaching discs (*d*), one of which is still in contact with a piece of stone (*st.*). (*s.*) = dormant shoots.

### *Reproduction.*

In *B. tuberculata* the oogonia and antheridia occur together in flask-shaped conceptacles, the oogonia occupying the base of the cavity and the antheridia the upper portion of the sides. Owing, however, to the later development of the oogonia young conceptacles contain only antheridia and appear to be unisexual.

Although many fresh plants with mature eggs and spermatozooids have been examined, only on two occasions was there any activity betrayed by the sexual organs; motile sperms were observed in July in material collected in Brittany, and very active spermatozooids have been found in material collected in April both in Guernsey and Plymouth. A curious parallel to this scarcity of recorded active sperms is found in the genera *Sargassum* and *Cystophyllum* in which Tahara (21) was unable to find any living spermatozooids.

#### (a) *The development of the conceptacle.*

The development of the surface depressions in which are produced the sex organs of the Fucaceae has long been a subject of discussion. Theories of conceptacle development are fully dealt with in recent papers by Simons (18) and Roe (17), amongst others. In the main previous observations have been interpreted in one of two ways:

- (a) According to Bower (2) and Roe (17) the conceptacle is initiated by a slight modification of the division of the segments formed from the apical cell, division in the longitudinal direction ceasing whilst the horizontal divisions persist giving a linear series of cells. Meanwhile

the surrounding cells divide as usual, so that the terminal cell of the series comes to lie in a groove. This latter cell (the 'initial' cell) eventually degenerates, the cell immediately below it (the 'basal' cell) contributing to the tissue lining the conceptacle, as also does the limiting layer and the cortical cells adjacent to the basal cell.

- (b) Simons (18) and Nienburg (14) are the chief exponents of the second theory of development. According to them, the true 'initial' cell of the conceptacle divides by a concave wall to give an upper 'tongue' cell and a lower 'basal' cell. From the latter the basal part of the conceptacle wall is eventually produced. The 'tongue' cell, which is presumably equivalent to Bower's 'initial' cell, may degenerate, or, as is more usual, give rise to a short filament. Thus the original initial cell *does* contribute to the growth of the conceptacle, for the true initial cell is represented by a modified epidermal cell *before* the latter becomes divided by the formation of a concave wall.

In *B. tuberculata* young developing conceptacles were best seen in longitudinal sections through very young fertile tips. Early stages in development were found to occur within the apical groove, or at the rim of the opening to the groove. The interior of the latter is so filled with mucilage that the detection of any slight irregularities in the epidermal layer, due to the beginning of conceptacle formation, was rendered difficult. For this reason the stage where the young conceptacle is represented by a single modified epidermal cell was not detected, although other later stages here figured suggest that the process of development is essentially that indicated by Nienburg for this species.

The initiation of a conceptacle is suggested in the first place by an interruption in the continuity of the lining layer of the groove, below which is visible a black streak—the mucilaginous tip of the initial cell of the conceptacle. Fig. 6*a* shows that the conceptacle can be regarded as arising from a complex of cells, whose origin from a single initial cell is strongly suggested. At this stage it will be seen that the initial cell has divided, first by a downwardly concave wall into an upper cell (which, after Simons, we may call the 'tongue' cell) and a lower basal cell. Moreover, the basal cell has already become divided longitudinally and transversely, thus obscuring the common origin of these cells from a single epidermal cell. The stage figured represents the tongue cell as almost conical in form, suggesting on comparison with Fig. 6*b* that this is due to the plane of cutting being slightly oblique. The base of this same cell is rendered conspicuous by the presence of a large nucleus, whilst the tip of the cell has already begun to swell. A separation of the cell wall of the apex of the initial cell from the walls of the cells surrounding it takes place at a very early stage, thus leaving the tongue cell free.



In a slightly older stage (Fig. 6 *b*) a similar complex of cells can be recognized, but here the basal cell has divided still further in a transverse direction, whilst the tongue cell shows no sign of degeneration, having now divided in two planes, thus initiating the formation of a hair.

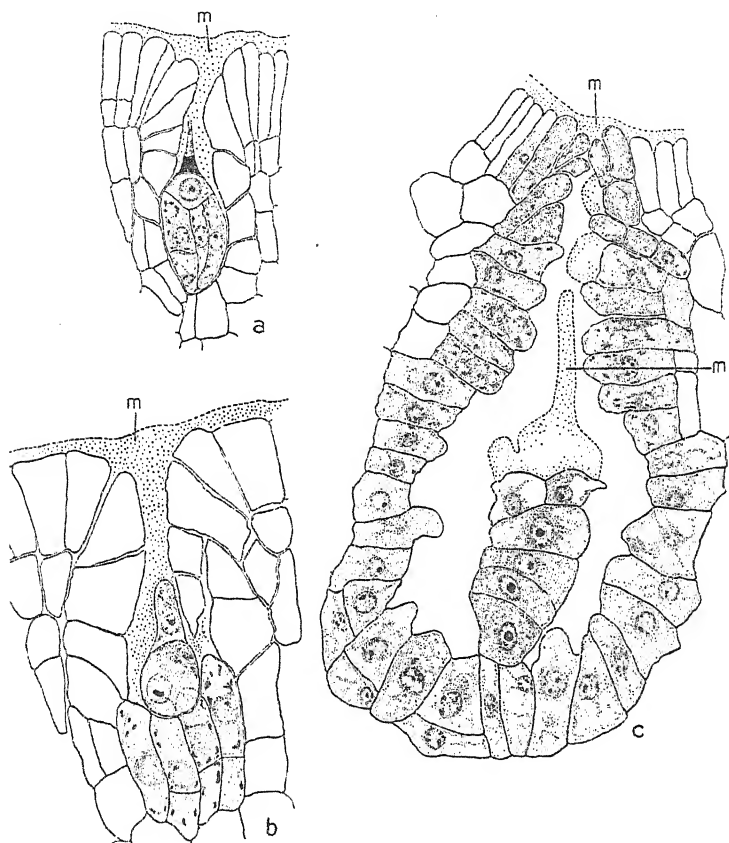


FIG. 6. *B. tuberculata*. V.S. through young conceptacles (with mucilage (*m.*)). (*a*) Showing complex of cells derived from single initial cell.  $\times 580$ . (*b*) Beginning of basal hair in a conceptacle slightly older than (*a*).  $\times 970$ . (*c*) Still older conceptacle with prominent basal hair.  $\times 500$ .

In a still older conceptacle (Fig. 6 *c*), situated at about 1 mm. from the tip of the erect shoot and on its outer surface, both the form of the conceptacle and the character of the basal hair have undergone a change. The former is no longer a narrow groove, but has become widened in its basal part by the lateral growth of the cells limiting this region. By this means the cavity assumes a flask-shaped appearance. The basal hair has become divided to give a conspicuous and irregular growth, whose terminal cells have degenerated, producing an apical mucilaginous mass. In other observed cases, the irregular appearance of the basal hair was still more

noticeable, the latter degenerating into a formless lump of tissue. In all the young conceptacles examined the presence of some structure of the nature of a hair was evident. This hair is, however, in no way comparable with the projections formed at the base of the young conceptacle in *Ascomphyllum*, since in *B. tuberculata* it is attached to the conceptacle wall by a narrowing base and obviously plays no part in determining the form of the conceptacle. Only when the sex organs and the paraphyses begin to form does the basal hair degenerate and finally disappear; even so, traces of the hair can still be seen in conceptacles where the male organs have formed.

Amongst the Fucaceae the connexion between hairs and conceptacles is best seen in the genus *Himanthalia*, where the first stage in the initiation of the conceptacle is represented by the appearance of hairs within the apical groove. In *B. tuberculata*, as in the rest of the Cystoseiro-Sargasseae, such hairs are now reduced to a minimum and are represented only by the tongue cell of the initial.

As far as conceptacle development is concerned, it appears therefore that the type found in *B. tuberculata* is more primitive than that found in *Fucus serratus* (where the upper half of the initial cell undergoes no divisions) and less so than in *Himanthalia*. The process in this species is most comparable with that described for certain species of *Sargassum* and *Cystoseira*, e.g. *S. filipendula* (Simons) and *Cystoseira barbata* (Nienburg).

(b) *The sexual organs.*

Within the young conceptacle paraphyses are the first to develop as papillate outgrowths of the inner conceptacle wall. Such papillae are visible in conceptacles in which the basal hair is still present. Certain of these papillae at a later stage produce the branched antheridial hairs, the tips of whose branches become transformed into antheridia typically Fucaeous in character. The antheridia, as is usual in this group, are small in comparison with the eggs and reach a length of about 30 to 40  $\mu$ . Stages in the formation of antherozoids were observed where the original nucleus of the antheridium had divided to give 2, 4, and 8 nuclei. There is, however, little doubt that the eight successive divisions of the antheridial nucleus occur, resulting finally in sixty-four spermatozoids.

While the conceptacle is still young, the whole of its wall is antheridial and there are no signs of the development of oogonia in the basal region. The oogonia make their first appearance in the basal region of slightly older conceptacles. At this stage the basal growth of the young conceptacle appears to move the antheridia upwards along the sides and in place of the antheridial hairs, the base of the conceptacle wall has now become occupied exclusively by the young oogonia with their accompanying unbranched paraphyses.

There seems to be no reason to believe that the development of the oogonium in *B. tuberculata* differs in any marked respect from that described for other genera of the Fucaceae, i.e. the oogonium arises from an outgrowth of one of the cells of the conceptacle wall and cuts off a small stalk cell. In this species only one egg is produced in each oogonium; this is not constant for the genus, since in *B. levigata* four eggs are produced in each oogonium (Kützing (8)). In *B. tuberculata* the younger smaller oogonia possess a single comparatively large nucleus lying centrally and provided with a large nucleolus. Older oogonia, on the other hand, show the presence of from 2 to 8 nuclei, which indicates that the three successive nuclear divisions take place. In some cases these nuclei, where their number exceeds four, are centrally placed, whilst in others they are peripheral; in a series of sections through a single oogonium, seven small peripheral nuclei could be distinguished together with one large central nucleus, the latter representing the nucleus of the egg. The supernumerary nuclei eventually degenerate, although no actual stages in degeneration were noted, for old eggs—which have a tendency to become pear-shaped—again possess a single nucleus, now greatly increased in size and often obscured by a densely staining mass of plastids.

The eggs in the oogonia of one and the same conceptacle do not develop simultaneously, some having reached the uninucleate stage whilst others still show the presence of several nuclei. This contrasts with the behaviour of the eggs in some of the Sargasseae, which mature and are liberated in acropetal zones along the receptacle.

*B. tuberculata* exhibits a feature of conceptacle construction seen in some species of *Sargassum*, namely, the formation of 'plugs' at the mouth of the conceptacle. These stopper-like organs begin to develop when the basal oogonial region of the conceptacle occupies about two-thirds of the conceptacle wall. The cells immediately surrounding the ostiole acquire a dark brown colour due to some change, probably a degenerative one, in the cell contents. The paraphyses in the upper region of the conceptacle also become darkly coloured and grow out into the cavity of the conceptacle forming a pseudoparenchymatous mass of dark cells, which completely blocks the entrance to the conceptacle. It not infrequently occurs that the major part of the antheridial region becomes involved in this blockage, so that the only recognizable part of the original conceptacle is the lower oogonial region. At a later stage, the plugs have disappeared and the conceptacle is left with a double cavity (Fig. 7), the upper part being lined with broken ends of cells. In old conceptacles where this has occurred, eggs are often found in the gap left by these plugs.

Material collected in April and July did not differ noticeably as to the presence of these structures. It is obvious that they are formed only in older receptacles, for in a series of cross sections through a receptacle whose

tip had proliferated into a new fertile growth, the old basal region abounded in plugs which were absent from the young apical region.

Since these stoppers develop at such a late stage in the history of the conceptacle, it seems unlikely that they should be concerned in the process of oogonial liberation, as is the case in those species investigated by

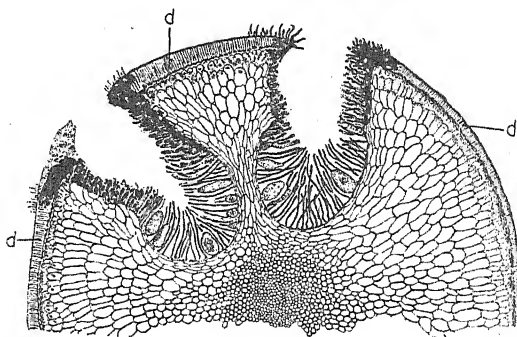


FIG. 7. *B. tuberculata*. X.S. of old receptacle, showing cavity left by 'plugs', also degenerate paraphyses in the neighbourhood of the ostiole.  $\times 35$ . *d* = mucilaginous degenerating cells.

Tahara (21). Probably the formation of these structures in *B. tuberculata* is entirely a process of degeneration, the mechanical shrinkage of the old receptacle pressing out this mass of degenerate and less resistant cells.

From a consideration of this single species of the genus *Bifurcaria*, there seems no reason to dispute the inclusion of the genus within the Cystoseiro-Sargasseae, as put forward by Oltmanns (16). Such a position, however, could only be tentative whilst the other two species remain undescribed.

#### SUMMARY.

1. The distribution, habitat, and method of growth of *B. tuberculata* Stackh. are discussed, with particular reference to the unusual development of a 'rhizome' in a member of the Fucaceae.
2. The anatomy of this species has been examined and the construction of the attaching disc described, the development of 'hyphae' being confined to this organ.
3. Stages in the development of the hermaphrodite conceptacle have been found and are figured in the foregoing account. The young conceptacle is characterized by the presence of a basal hair which persists up to the time of formation of the sexual organs. In the conceptacles, the oogonia and antheridia are confined to the base and upper regions of the conceptacle respectively. Each oogonium, the nucleus of which undergoes the usual three successive nuclear divisions, produces a single egg. The seven supernumerary nuclei formed degenerate after passing to the peri-

phery of the egg, and a single central nucleus is left—this being the nucleus of the oosphere.

4. In old receptacles, the mouths of the conceptacles become stopped up by the growth of paraphyses in the neighbourhood of the ostioles. Later, the pseudoparenchymatous mass thus formed breaks away as a plug-like structure, leaving the conceptacle with a double cavity.

5. It is concluded that the affinities of *B. tuberculata* lie, as decided by Grüber, with genera such as *Cystoseira*, *Halidrys*, and *Sargassum*, and this species is probably rightly placed in the Cystoseiro-Sargasseae.

In conclusion, I wish to express my thanks to Dr. E. M. Delf for much encouragement and helpful criticism during the progress of this investigation, and to Dr. V. M. Grubb for assistance in preparing this paper for publication.

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# Contributions to our Knowledge of the Myxophyceae of India.<sup>1</sup>

BY

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With eight Figures in the Text.

1. *On a Form of Cylandrospermum with Heterocysts at both ends of the Filaments* (*Cylandrospermum muscicola* Kütz. var. *kashmirensis* var. nov.)<sup>2</sup>

THE alga, forming the subject of this communication, was collected in May, 1931, from a shallow pond in Srinagar, Kashmir, at a height of 5,190 feet above sea-level, growing as an epiphyte on *Myriophyllum*. It forms a thin, velvety, shining, bluish-green irregular layer, very soft and mucilaginous to the touch, attached closely to the substratum; the individual strata are of limited extent, the maximum width of a stratum being about 1.5 cm. The mucilaginous mass comprises many layers of unbranched filaments, which in the upper part of the stratum are more or less straight and parallel and form a compact layer, whilst in the lower part adjacent to the substratum they are irregularly bent and more or less entangled.

The blue-green trichomes are of considerable length (from  $\frac{1}{8}$  to  $\frac{1}{2}$  mm. long) and are slightly constricted at the joints. The cells are more or less barrel-shaped, and may be twice as long as broad, though occasionally length and breadth are equal; the dimensions are 2.6–3.9  $\mu$  broad and 2.6–8.4  $\mu$  long. The septa are very distinct. When a filament is stained with an alcoholic solution of iodine, the longitudinal walls, representing the outer investments of the cells, become dark brown and appear moderately thick (Fig. 1, B and C, *o*). The longitudinal walls of adjacent cells are interrupted by the transverse septa which remain colourless (Fig. 1, B and C, *i*). Internal to the outer investment lies a narrow colourless strip, not stained with alcoholic iodine; this is continuous with the septum and represents the inner investment (Fig. 1, B and C, *i*). The two envelopes

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<sup>2</sup> Latin diagnoses of the new forms described in this paper will be published elsewhere.

of the cells thus show the features described by Fritsch (11) in *Anabaena*, and later by Spratt (24) in *A. Cycadeae*. After solution of the cell-contents with 33 per cent. chromic acid the walls exhibit considerable contraction.

Granules, which are angular in the preserved material, are always found in the peripheral cytoplasm; they are larger in old than in young cells and specially large in the spores. The cell contents take up all the stains mentioned by Geitler (16) and Olive (22). Long immersion in aqueous methyl green, however, turns the cell contents violet. Alcoholic eosin stains the cytoplasm lightly and the granules very deeply (cf. Fritsch (10) and Spratt (24)). Alcoholic brilliant green colours the cytoplasm without affecting the granules. The granules disappear in Eau de Javelle, dilute acids and caustic potash.

A single heterocyst is situated at each extremity of the mature filament (Fig. 1, A, *h.*), although in some of the younger filaments there may be a heterocyst only at one end. The heterocysts are oval or ellipsoidal (Fig. 1, B, D-G) and have homogeneous contents of a greyish colour in the preserved material. They are  $3.9-5.2\ \mu$  broad and  $5.2-10.5\ \mu$  long, and are provided with two walls.

In the development of a heterocyst from an ordinary vegetative cell, the large granules first disappear and the cell contents gradually become homogeneous. At the same time the cell enlarges and its inner investment thickens and separates from the outer on the side adjoining the next vegetative cell, where a shorter or longer pore is formed (Fig. 2, A). Through this pore communication between the developing heterocyst and the adjoining vegetative cell is maintained. Before the granules disappear the two envelopes of the developing heterocyst are sometimes clearly visible (Fig. 2, B), the inner one being somewhat thickened, most markedly around the pore. The relation of these two envelopes with those of the vegetative cell is not clear. The outer wall also thickens, and in mature heterocysts becomes equally prominent. After long immersion in chlorzinc iodide the inner wall gives the violet reaction of cellulose (cf. Geitler (13)) and the pore stands out clearly. According to Geitler (13, p. 227) the outer wall of the heterocyst is of a pectic nature, but I have failed to obtain the reaction for pectin substances when my material was treated with ruthenium red (cf. Tunmann (25)). At a later stage there appears opposite the pore a bright refractive granule which seems to enlarge as the heterocyst matures, so that when the pore has closed through the thickening of its walls a large angular granule lies close behind it (Fig. 1, D and E, *g.*), occasionally a little to one side. These granules show the same reactions as those found in the vegetative cells. In some cases a few further granules, apparently of the same nature, have been observed (Fig. 1, E, *g'.*). In heterocysts adjoining mature spores there are no granules (Fig. 1, F and G). In some cases the contents of the mature heterocysts had contracted away



from the inner wall at the distal end (Fig. 1, F and Fig. 2, C), but this may have been due to the action of the preservative. The contents assume a deep colour after long immersion in aqueous methyl green or in Heiden-

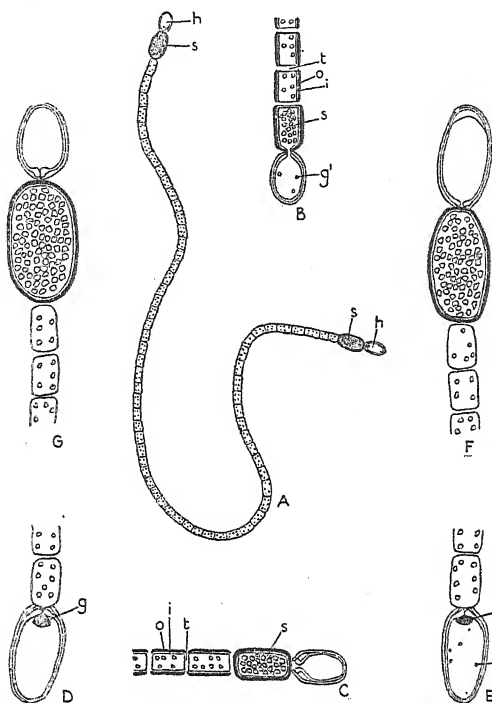


FIG. 1. *Cylindrospermum muscicola* Kütz. var. *kashmirensis*, var. nov. A, mature filament with heterocysts and spores; B and C, portions of filaments stained with iodine, showing the investments of the cell and the development of the spore; D and E, mature terminal heterocysts; F and G, heterocysts with mature spores. *g*., refractive granule of heterocyst; *g'*., other granules; *h*., heterocyst; *i*., inner investment; *o*., outer investment; *s*., spore; *z*., septum. A  $\times 440$ ; B-G  $\times 1,475$ .

hain's haematoxylin. In some cases the heterocysts were surrounded by a layer of mucilage which stained with methylene blue and bismarck brown. To this mucilage were attached a number of rod-like bacteria either singly or in linear chains (cf. Borzi (3)).

Intercalary heterocysts, which either occur singly (Fig. 2, D and E, *h*.) or in pairs (Fig. 2, G, *h*.) and are always in an early stage of development, are sometimes found in the middle of a filament. Such heterocysts develop from vegetative cells in the way just described. When these heterocysts occur in pairs, they are usually shorter than the ordinary vegetative cells and appear to have originated from a cell (Fig. 2, F) which has recently divided. Elongate incipient intercalary heterocysts, with a delicate transverse septum dividing them into two (Fig. 2, F, *h*.), are occasionally observed, and it is possible that pairs of intercalary heterocysts always arise in this way, viz. by division of a heterocyst rudiment. At

a time when the young heterocyst only differs from a vegetative cell in the paucity of granules and the homogeneous character of the cell contents, but before the wall thickens it rounds off on one side and the filament breaks into two pieces, one or both of which are thus terminated by a heterocyst. Further development of the young heterocysts takes place after the filaments have separated into two. In no case have fully developed heterocysts been observed in the middle of a filament.

When mature, these originally intercalary heterocysts resemble in all respects the terminal ones. Filaments with young intercalary heterocysts are rather rare, but those with a mature heterocyst and an adjacent spore at one extremity and a young undeveloped heterocyst or merely a vegetative cell, as the case may be, at the other are easily found. Filaments terminated at one end by a vegetative cell may either have resulted from the splitting of filaments with a single median heterocyst or by the dying of an occasional vegetative cell (Fig. 2, H, *d.*) in the middle of a filament. Wołoszyńska (27) states that the terminal heterocysts in *Anabaena circularis* G. S. West var. *javanica* Wołosz. (*Anabaenopsis circularis* (G. S. West) Wołosz. et Miller var. *javanica* Wołosz.) are originally intercalary, their terminal position being due to fragmentation of the filaments. As just explained, this is also the case in the alga under discussion.

Spores are produced later, and frequently one adjoins the terminal heterocyst at either end of the filament (Fig. 1, A, *s.*). Spores may, however, be formed only at one extremity, without any trace of spore-development at the other. The spores are larger than the heterocysts, being cylindrical when young (Fig. 1, C, *s.*) and either barrel-shaped or ellipsoidal when mature (Fig. 1, F and G). The mature spores measure  $5.2-7.8\ \mu$  broad and  $9.4-13.6\ \mu$  long. Each spore has two walls, the outer very thick, smooth and brown in colour, the inner thin and transparent. The two walls are developed from the two investments of the vegetative cell from which the spore differentiates (Fig. 1, B, *s.*). The contents are bluish-green with numerous large granules which occupy the whole protoplast.

During the early stages of spore-development the wall adjacent to the heterocyst exhibits a distinct pore, by means of which the contents of the sporogenous cell communicate with those of the heterocyst, whose canal-like pore is wide open (Fig. 2, C, *s.*). The granules in the cytoplasm of the sporogenous cell increase in number till they fill the whole cavity. At the same time the outer investment gradually closes in round the open ends and ultimately forms a complete envelope round the mature spore (Fig. 1, B, *s.*). As this happens, part of the transparent intercellular septum is enclosed to form the inner wall at the ends of the spore. The communication between the developing spore and the heterocyst is thus closed (Fig. 1, C, *s.*). The outer wall gradually undergoes uniform thickening, except on the side remote from the heterocyst, where the thickening takes place a little later

(Fig. 1, B, s.). The development of the spores is thus in close agreement with that described by Fritsch (11) for *Anabaena*, except for the delayed thickening of the wall on the side remote from the heterocyst. The spores do not contain any oil, as treatment with osmic acid gives no black colour.

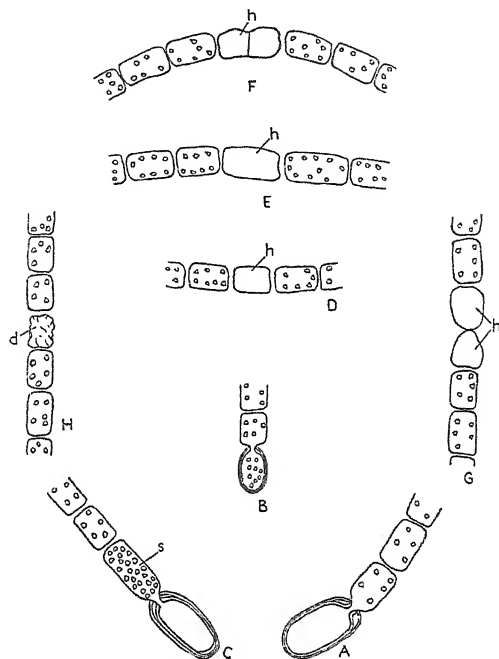


FIG. 2. *Cylindrospermum muscicola* Kütz. var. *kashmirensis* var. nov. A and B, young terminal heterocysts; C, development of spore; D and E, intercalary heterocysts; F, formation of a pair of intercalary heterocysts by the division of a heterocyst-rudiment; G, pair of intercalary heterocysts about to separate; H, filament with a dead cell. *d.*, dead cell; *h.*, heterocyst; *s.*, spore. All  $\times 3,475$ .

This alga resembles *Cylindrospermum* in possessing terminal heterocysts, each with an adjoining spore, but it differs in the usual presence of a heterocyst at each end of the filament. This last character is distinctive of the genus *Anabaenopsis*, established in 1923 by Miller (21) who raised Wołoszyńska's section *Anabaenopsis* of *Anabaena* to generic rank. *Anabaenopsis* includes those anabaenoid forms which exhibit a terminal heterocyst at each end of the filament. Miller included four species in this new genus: viz. two African ones described by West (26), *A. circularis* (G. S. West) Wołosz. et Miller (*A. flos-aquae* (Lyngb.) Bréb. var. *circularis* West) and *A. Tanganyikae* (G. S. West) Wołosz. et Miller (*A. Tanganyikae* West); one from Java, *A. Raciborskii* Wołosz.; and a Russian species, *A. Elenkini* v. Miller. The genus is recognized by Frémy (8) and Geitler (14, 15, 16); the latter (16) includes three further species, namely *A. Nadsonii* and *A. Milleri* described by Woronichin from Siberia and *A. Arnoldii* described

by Aptekarj from Russia. The alga here described resembles *A. Arnoldii* and *A. circularis* var. *javanica* in the presence of intercalary heterocysts, though they do not mature until the fragmentation of the filament is accomplished.

Frémy gives as the distinguishing characteristics of *Anabaenopsis*, apart from the terminal heterocyst at each end of the filament, the development of the spores remote from the heterocysts and the usual circular or spirally coiled form of the trichomes. Geitler (16) similarly describes *Anabaenopsis* as possessing trichomes, mostly coiled in the form of a spiral or a screw, rarely straight, and spores remote from the heterocysts. The coiled form of the trichomes is, however, not general for *Anabaenopsis*, since in *A. Raciborskii* the trichomes are usually straight and in *A. circularis* occasional straight ones are met with. Moreover, coiled trichomes are met with in quite a number of species of *Anabaena* (e.g. *A. spiroides* Klebahn, *A. Bolochonzevii* Meyer). Similarly, the position of the spores cannot be taken as a general characteristic of *Anabaenopsis*, since spores are unknown in one species and are only known in a variety of another. Intercalary spores remote from the heterocysts and either occurring singly or in pairs, moreover occur in many species of *Anabaena* (e.g. *A. elliptica* Lemm., *A. macrospora* Klebahn, &c.). The only valid distinction between *Anabaenopsis* and *Anabaena* therefore lies in the presence of heterocysts at the ends of the filaments.

The species of *Cylindrospermum* usually have a heterocyst only at one end of the filament. Glade (18), in *C. minutissimum* Collins, has, however, recorded filaments with heterocysts at each end, while Drew (7) has described similar cases in *C. licheniforme* Kütz. and *C. maius* Kütz., spores sometimes adjoining each terminal heterocyst in her material. Glade's material had been grown in culture solutions, while that of Drew had been kept in a damp chamber for a few days. Their forms thus occurred under artificial conditions and could therefore be regarded as exceptional cases, were it not that Frémy (9) has recently in material collected from natural habitats recorded heterocysts at both ends of the filaments, not only in *C. maius* Kütz. and *C. licheniforme* Kütz. but also in *C. catenatum* Ralfs. Brühl and Biswas (5) likewise report the finding of such cases in a *Cylindrospermum*<sup>1</sup> from a natural habitat, while Borge (2) has described two African varieties (*C. Goetzei* Schmidle var. *binum* and *C. muscicola* Kütz. var. *variabilis*) in which heterocysts normally occur at both ends of the trichomes, although in the second case this is only an occasional condition. Borge points out that this recalls *Anabaenopsis Raciborskii* and remarks that, if Geitler's (15) diagnosis of *Anabaenopsis* be followed, the two plants referred by him to *Cylindrospermum* would have to be included in the

<sup>1</sup> They describe the form as *C. doryphorum* Brühl and Biswas, but, as Geitler (16, p. 814) points out, so inadequately that the species must be dropped.

former genus. Owing to the position of the spores adjacent to the heterocysts Borge, however, adopts a reference to *Cylindrospermum*. About one-third of the described species of *Cylindrospermum* are thus at the present time known to be capable of occasionally forming heterocysts at both ends of their filaments. It would, however, scarcely be justifiable to create another new genus on this basis alone for the accommodation of these species.

Skuja (23), moreover, has in *Anabaena echinospora* Skuja recorded both intercalary and terminal heterocysts in the mature filaments, while the young ones have heterocysts only at the two ends. Heterocysts may thus be developed at both ends of a filament, both in *Cylindrospermum* and *Anabaena*. In view of these facts *Anabaenopsis* can scarcely stand as a separate genus, since the only valid feature, the presence of a heterocyst at either end of the filament is one that can occur both in anabaenoid and in *Cylindrospermum*-types. It would consequently seem best to discard the genus *Anabaenopsis* and to re-establish the section *Anabaenopsis* (as distinct from the section *Euanabaena*) of *Anabaena* to include those species of the latter which possess heterocysts at both ends of the filaments. In the same way a section *Cylindrospermopsis* of *Cylindrospermum* might be created for the Indian form here described and other species of the genus with heterocysts at both ends.

According to the key given by Geitler (16) the alga described above agrees in essentials with *C. muscicola* Kütz., except in the blue-green colour of the thallus, the more or less barrel-shaped comparatively narrow vegetative cells which are longer than broad, the oval or ellipsoidal heterocysts which are larger, and the ellipsoidal or barrel-shaped spores which are much smaller. The epiphytic habit of the Indian plant is also very distinctive. The alga is thus a new variety of *C. muscicola* Kütz. which may be named var. *kashmirensis*.

## 2. Formation and Germination of Spores in *Aulosira Fritschii* sp. nov.

The alga described below was found in July 1931, together with certain other algae, on dead leaves that had fallen into the water of a shallow pond near the Benares Hindu University grounds. The alga itself bore numerous epiphytes, including narrow species of *Oedogonium* and *Bulbochaete*, as well as unicellular forms. The material, on which the following description is based, was preserved on the spot in weak formalin. The pond contained plenty of water, and there was no reason to assume that the alga was approaching the end of its period of abundance.

The thallus forms a dirty blue-green or dark green woolly felt-like covering on the surface of the leaves. It consists of unbranched filaments which reach a length of about 7 mm. and are more or less loosely

entangled; the filaments are straight or occasionally irregularly bent. The blue or bluish-green trichomes are constricted at the joints and enclosed in a thick sheath in which two portions can be distinguished (Fig. 3, A-C). The outer hyaline portion (*o*) is stratified with parallel layers, its outer edge being irregular and diffuent. The stratification is clearly brought out by Heidenhain's haematoxylin. This outer sheath is well defined in the purely vegetative trichomes, but in the sporogenous ones it becomes more diffuent and partly dissolved (Fig. 3, D; Fig. 4, C, D, and F, *o*). The inner sheath is of a denser consistency and rather thinner, unstratified, and of more or less uniform thickness (Fig. 3, A-C, *i*), except around the spores where it is generally compressed and appears very thin (Fig. 4, A-C, *i*), sometimes being only recognizable as a faint line (Fig. 3, E and F, *i*). In such filaments, however, it exhibits annular ingrowths, conical in optical section, which occur opposite the constrictions between adjacent spores (Fig. 3, D-F; Fig. 4, A-E, *a.i.*). The maximum thickness of the double sheath is  $3.1\ \mu$ . In the vegetative trichomes both the inner and outer sheaths are firm and retain their form, when the contained cells die (Fig. 3, A; Fig. 4, G). The sheath readily takes up all kinds of stains (aqueous methyl green, ruthenium red, alcoholic safranin, &c.), the inner sheath in all cases staining deeper than the outer. These reactions show an affinity of the sheath for basic aniline dyes and indicate its pectic nature (cf. Lemaire (19) and Geitler (16)). Chlor-zinc iodide stains the sheath violet, with or without previous treatment with Eau de Javelle, and this shows that it also contains cellulose, probably in a combined state (cf. Lemaire (19)). The sheaths of sporogenous trichomes stain less deeply than those of the purely vegetative ones. There is generally a narrow space between trichome and sheath.

The cells are  $8.4\text{--}10.5\ \mu$  in diameter, generally slightly longer than broad, but sometimes quadratic in optical section; more rarely they are elongate, two, three, or even four times as long as broad. The cells on either side of the heterocyst are generally drawn out towards it in a characteristic way (Fig. 3, B and C). A similar feature has been recorded by Carter (6) in *Microchaete uberrima* Carter and by Lemmermann (20) in *Aulosira Schauinslandii* Lemm. The cell-walls are of some thickness. The cell contents are blue or deep blue-green and finely granular. They show practically the same reactions to various stains and reagents as in the *Cylindrospermum* above described. With Heidenhain's haematoxylin the stain is lighter, but the granules become very prominent.

The heterocysts are single and intercalary (Fig. 3, B and C), generally cylindrical or rarely elongate-ellipsoidal in form and usually slightly longer than broad, or sometimes about twice as long as broad; the dimensions are  $9.4\text{--}11.5\ \mu$  broad and  $14.7\text{--}23.5\ \mu$  long. A single heterocyst was found which was four times as long as broad ( $9.4\ \mu \times 37.6\ \mu$ ). Young heterocysts

have deep blue-green, rarely light violet, finely granular contents like those of the vegetative cells, but in the mature ones the contents become vacuolated and assume a yellow or orange colour, although the granules remain as before. The heterocysts extend right up to the inner sheath with which they are in close contact. The outer wall is thick, while the inner one is thin, being clearly evident in those rare cases in which it has receded from the outer one (Fig. 3, c). The wall around the distinct terminal pores is not specially thickened. The conspicuous granules internal to the pores sometimes reach a considerable size in old heterocysts (Fig. 4, G, *g.*). They are sometimes of irregular shape, though often either plano-convex or concavo-convex, with the convex side away from the pore. Vacuolization commences rather early, so that by the time a heterocyst has reached its full size it possesses a large central cavity with the granular contents occupying the periphery. The oldest heterocysts show practically no contents (Fig. 4, D and G, *h.*). The outer wall of the heterocyst is very persistent, being evident at regular intervals in old filaments in which the vegetative cells have perished. The contents of the young unvacuolated heterocysts stain much more deeply with 1 per cent. aqueous methylene blue than do those of the vegetative cells, but the outer wall is not affected. The cellulose inner wall stains violet with chlor-zinc iodide. With Ehrlich's haematoxylin the granules adjacent to the pores assume a dark colour like those in the vegetative cells.

The *spores* are formed centrifugally in long chains, their formation commencing about midway between two heterocysts and gradually advancing towards them. In one filament sixty-five spores occurred in a continuous chain between two heterocysts, but usually the latter are much nearer to each other so that the chains of spores are considerably shorter. Not uncommonly they are interrupted by vegetative cells. Sometimes the whole of a long filament may consist solely of spores, with intervening heterocysts at more or less regular intervals. The spores are usually roughly quadratic in optical section, varying between  $10.5\ \mu$  and  $12.6\ \mu$  in width. Some are cylindrical, up to twice as long as broad.

Each spore has a thick pale brown exospore and a thin hyaline endospore (Fig. 3, D-F, *Ex.*, *End.*); in the preserved material the latter had, in most cases, contracted slightly away from the former. In the mature condition the adjacent parts of the exospore of adjoining spores are closely adpressed to each other, and the appearance is obtained as though all the spores of a chain were joined together and inseparable. Even when sporogenous filaments were placed in strong sulphuric acid for more than a fortnight and subjected to subsequent teasing, the spores could not be separated. The mature exospore, though uniformly thickened over most of its surface, exhibits a special thickening at the corners, which is more marked towards the inside than towards the outside; as a result the inner

surface of the membrane at the four corners is protruded, giving the inner contour of the spore a somewhat octagonal outline in optical section (Fig. 3, D-F). In the contents of the mature spores one can distinguish an extensive central region containing large refractive angular granules and a peripheral region of varying width occupied by fine granules, like those found in the vegetative cells (Fig. 3, D-F, *l.g.*, *f.g.*). The mature spores are thus characterized by the thick closely adpressed transverse walls, the sub-octagonal contour of the contents and the large granules occupying the greater portion of the latter.

Stained with 1 per cent. aqueous methylene blue the exospore becomes light blue, and is difficult to distinguish from the similarly coloured inner sheath which is closely adpressed to it. In the same way alcoholic safranin and aqueous ruthenium red stain the exospore red. The contents, including the granules, are unaffected by any of these stains, as well as by eosin. On the other hand, methyl green colours the exospore light violet, while the inner sheath becomes blue, and thus the latter stands out clearly between the exospore and the outer sheath which also stains violet. With iodine reagents the contents of a mature spore become brown or yellowish-brown, the granules appearing slightly darker. The granules are dissolved by Eau de Javelle, potassium hydroxide, and dilute acids. There appears to be no fat present. In chlor-zinc iodide or strong salt solution the contents of most of the mature spores contract into a dumb-bell shaped mass; the cause of this contraction is not apparent.

The spores develop from the vegetative cells by increase of size and enlargement of the granules, while the colour of the contents changes to light blue-green or yellowish-green. The walls thicken and the exospore and endospore soon become distinguishable. The immature spores thus formed are light blue-green or yellowish-green with a thick pale brown exospore, while large granules are scattered throughout the contents; the walls of adjoining spores appear separated at their middle, while in contact at their edges (Fig. 4, A). At a slightly later stage the inner contour of the spore-wall becomes somewhat protruded at the corners (Fig. 4, B and F) and subsequently, when the contents may acquire a yellow colour, they differentiate into a central region with large granules and a peripheral zone with minute granules; the adjoining walls of adjacent spores are still recognizable as separate entities (Fig. 4, C). This leads over to the mature state in which the inner contour of the spore-wall shows the typical form, and the adjoining walls between the spores are so closely adpressed to each other throughout their length that they appear to form a common transverse septum (Fig. 3, D-F, *z.*).

Certain of the unripe spores of various ages show vacuoles in their contents, and these are very distinct at the stage when only small granules are present (Fig. 4, A, *v.*). The vacuoles are at first small and gradually



merge to form larger ones. Short chains of unripe spores exhibiting these phenomena have been commonly observed. In most of these cases vacuolization proceeds to such an extent that practically the entire contents

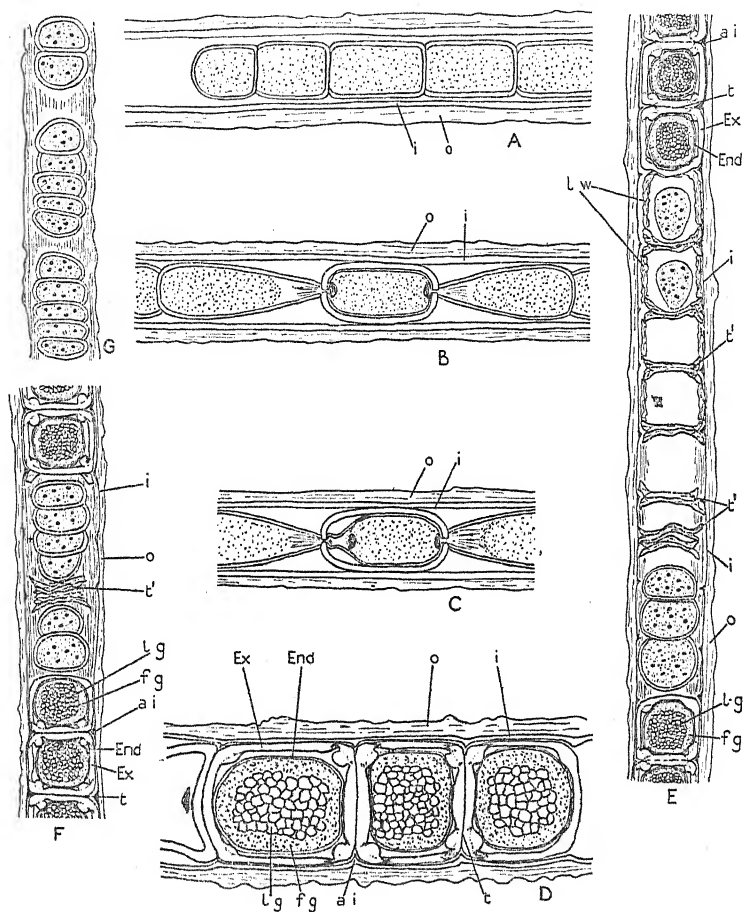


FIG. 3. *Aulosira Fritschii* sp. nov. A, portion of a filament; B and C, filaments with intercalary heterocysts; D, filament with mature spores; E and F, germination of mature spores; G, three new trichomes formed by the germination of mature spores, embedded in a linear series within a tubular mass of mucilage; *a.i.*, annular ingrowth of the inner sheath opposite the constrictions between adjacent spores; *End.*, endospore; *Ex.*, exospore; *f.g.*, fine granules; *i.*, inner sheath; *l.g.*, large granules; *l.w.*, longitudinal diffuent walls of germinating spores; *o.*, outer sheath; *t.*, common septum between adjacent spores; *t'*, septa left by dead spores. A-C and E-G  $\times 685$ ; D  $\times 1,475$ .

are obliterated and such spores ultimately degenerate and die (Fig. 4, A and B, *d.s.*). After the contents have died the transverse walls become dissolved, while the longitudinal walls remain intact for some time, so that such stretches of a filament appear as a hollow tube, the wall of which consists of the two sheaths and of the longitudinal walls of the dead spores

(Fig. 4, A, *h.t.*). The solution of the transverse wall starts at its centre and proceeds towards the periphery and generally leaves a narrow peripheral strip attached to the longitudinal walls. Such strips appear as ring-like ingrowths jutting in from the longitudinal walls into the interior of the dead filament (Fig. 4, A and E, *i.d.s.*). Ultimately both the longitudinal walls and the ring-like remains of the transverse walls gelatinize and gradually disappear (Fig. 4, F and G), the latter vanishing much earlier than the former.

Not uncommonly mature spores degenerate in the same way, but in this case only the longitudinal walls of the dead spores are transformed into mucilage, whilst the transverse septa persist. By such dying away of spores gaps are formed which make it possible for other spores in the filament to germinate *in situ* (Fig. 3, E). The transverse septa, left by dead mature spores, are then gradually pushed forwards, as new trichomes grow out from germinating spores (Fig. 3, E, *l.*).

In the first stages of the germination of a spore the longitudinal walls of the exospore lose their pale brown colour and gradually become diffuent; at this stage they present a wavy appearance and become deeply stained with 1 per cent. aqueous methylene blue (Fig. 3, E, *l.w.*). Later the endospore also becomes mucilaginous. The mucilage thus formed around the germinating spore shows parallel strata and is coloured violet by methylene blue. The end-walls, although they become more or less shrivelled and deformed, retain their pale brown colour and remain in their places. In the meantime the contents of the spore lose the majority of the large granules and become finely granular like the vegetative cells, although a few larger granules are to be seen here and there. In this condition the spore contents are stained with methylene blue like those of the vegetative cells. The protoplast now commences to lengthen parallel to the long axis of the filament, and becomes divided by septa to form a short trichome of two or more cells, the walls of which soon thicken. As the young trichome grows, it pushes the pale brown deformed end-wall of the spore, as well as the persisting end-walls of disintegrated spores, in front of it, and the number of the latter indicates the number of original spore-cavities which it has occupied during its growth (Fig. 3, E, *l.*). When two such trichomes develop from opposite sides, at some little distance away from one another, they gradually push the persisting remnants of the transverse walls together into a central group (Fig. 3, F, *l.*). Later, the displaced remnants of the transverse walls become transformed into mucilage and dissolved. The two sheaths of the original trichome also become mucilaginous and the new trichomes, which are then usually from 2-5 cells in length, are found embedded in a linear series within a tubular mass of mucilage (Fig. 3, G) in which they continue to lengthen with the formation of new sheaths till they become typical filaments.

The spores of this plant germinate soon after they are mature, although even immature spores may germinate (cf. below). There is there-

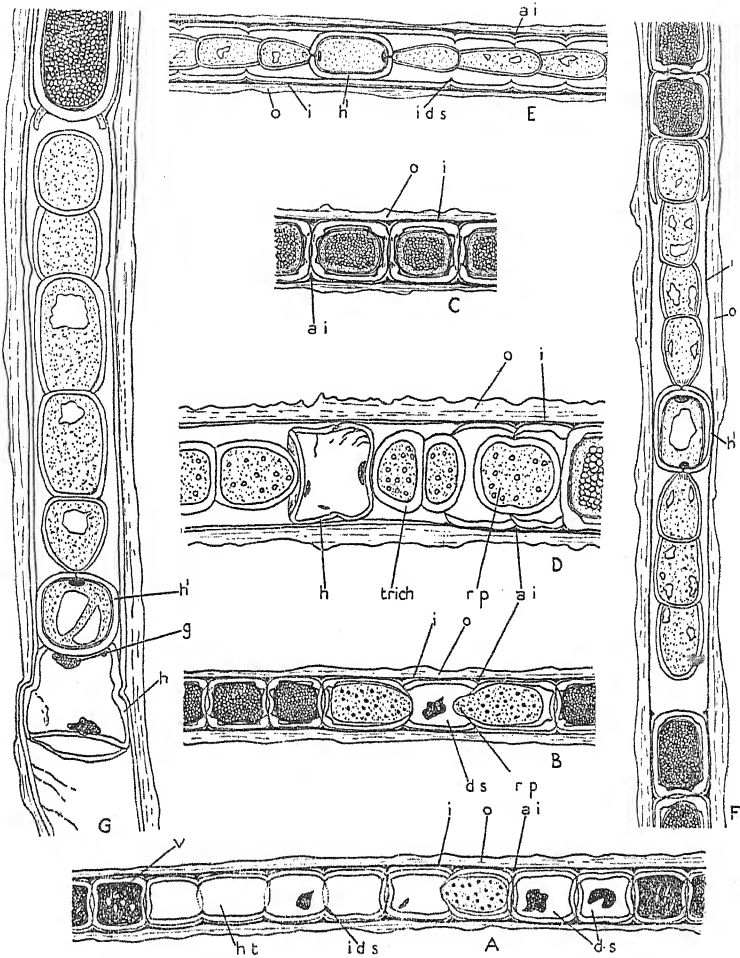


FIG. 4. *Autoliria Fritschii* sp. nov. A and B, filaments with living spores and various stages in their degeneration, also germinating spores; C, filament with immature spores, the end walls of adjacent spores still recognizable; D, germination of immature spores, like those in C; E, new trichome formed by the germination of an immature spore and surrounded by the persisting longitudinal walls of disintegrated spores and the sheaths of the parent-filament; F and G, new trichomes formed by the germination of immature spores within the sheaths of the parent filaments. *ai*, annular ingrowth of the inner sheath; *ds*, dead spore; *g*, granule; *h*, heterocyst of parent-trichome; *h'*, heterocyst of new trichome; *h.z.*, tube formed by the death of the contents of immature spores; *i*, inner sheath; *i.d.s.*, peripheral strips of incompletely dissolved septa; *o*, outer sheath; *r.p.*, rejuvenated protoplast; *trich.*, trichome; *v*, vacuole. A-C and E and F  $\times 685$ ; D and G  $\times 1,475$ .

fore, so far as my material shows, no resting period. Similar cases have been described by Fritsch (12) and Bristol (4). Since the end-walls of the spores at first remain unaltered, we have a partial gelatinization of the

spore-walls, such as has been reported by Fritsch in *Anabaena Azollae* (loc. cit., Fig. 6). The germination of a number of spores *in situ*, with the production of a linear series of young trichomes embedded in mucilage, recalls the condition figured by Fritsch (loc. cit., Fig. 11).

Quite frequently immature spores likewise germinate *in situ*, forming short trichomes which grow into a gap created by the death of one or more of such immature spores. In germination the contents of the immature spores undergo the same changes as were above described for the mature ones and the endospore becomes mucilaginous. That half of the common transverse wall, which belongs to the adjoining dead spore, becomes dissolved, while the other half becomes protruded into the adjoining space (Fig. 4, A and B). The central part of this protuberance becomes ruptured, and through the aperture the rejuvenated protoplast emerges (Fig. 4, B, *r.p.*), usually appearing compressed at the point of exit, although sooner or later the aperture widens and the narrowing of the protoplast disappears (Fig. 4, D, *r.p.*). This method of germination agrees with observations of Borzi (3), Fritsch (12), and Spratt (24) (cf. Fritsch, Fig. 25, and Spratt, Fig. 17). As soon as it emerges, the protoplast becomes divided by a septum and forms a trichome of two cells (Fig. 4, D, *trich.*), which, provided sufficient space is available, may grow into a fairly long thread. In such cases the trichomes are enclosed in a tubular envelope composed of the two sheaths and the undissolved longitudinal walls of the disintegrated spores, bearing as ingrowths the incompletely dissolved transverse septa which usually in no way correspond in position to the septa of the new trichome (Fig. 4, E). The appearance thus obtained is highly peculiar, since the envelope of the trichome bears at regular intervals annular ingrowths which, without a knowledge of their mode of origin, would be regarded as part of the sheath. As previously stated, the incompletely dissolved transverse septa ultimately get dissolved, and sooner or later they are followed by the longitudinal walls of the disintegrated spores (Fig. 4, F and G).

Heterocysts are formed quite early (cf. Fritsch (12) and Bristol (4)), being found usually already in trichomes of only 6–8 cells (Fig. 4, F and G, *h.*). These heterocysts in the young trichomes are generally intercalary (Fig. 4, E and F, *h.*), sometimes terminal (Fig. 4, G, *h.*). The trichomes originating from immature spores always remain inside the sheaths of the parent-filament and, though they sometimes attain considerable length, they appear not to live long as vacuolization commences at an early stage, both in the cells and heterocysts. Even in short trichomes of a few cells only, practically all the cells and heterocysts exhibit vacuolization and the contents usually show signs of degeneration. In long trichomes, which are generally found at the ends of the parent-filaments, a number of the terminal cells often assume a rounded shape, but such cells are frequently

practically devoid of contents. When such a long trichome, formed either in the middle of a filament or at one end, possesses a terminal heterocyst adjoining an empty space, the parent-filament sometimes breaks away from the heterocyst, so that the trichome enclosed within the sheaths of the parent-filament is separated off.

The mode of persistence of this alga thus remains obscure. It is probable, however, that some of the spores do not germinate *in situ*, but survive after the death of the rest and lead to a fresh development during the next period of activity.

This alga undoubtedly belongs to the genus *Aulosira* of the family Microchaetaceae, as shown by the unbranched filaments, without differentiation between base and apex, the intercalary heterocysts and the thick firm sheath. It does not, however, agree with any of the species so far described, and there are several respects in which it differs from all previously established species of the genus. These are the double sheath, the presence of granules in the contents of even old heterocysts, the remarkable thickening in the corners of the exospore of the mature spore, and the degeneration of a large number of spores at an earlier or later stage combined with germination of others *in situ*. Like *Aulosira implexa* Born. et Flah., *A. fertilissima* Ghose, and *A. africana* Frémy, the spores are formed in chains, but their shape and dimensions are different to those of any of these species; *A. implexa* and *A. fertilissima* have elongate-ellipsoidal spores with rounded ends, while those of *A. africana* are slightly constricted in the middle. Nor do any of these species develop the remarkably thick spore-envelopes of the form here described. The threads are never associated in bundles, as is characteristic of *A. implexa*. The mode of occurrence on dead leaves is similar to that of *A. fertilissima*.

The alga must therefore be regarded as a new species to be named *Aulosira Fritschii*, sp. nov.

### 3. On a Species of *Aulosira* (*A. prolifica* sp. nov.) exhibiting only Vegetative Reproduction.

In August, 1931, a dense mucilaginous scum formed on the surface of the water of a shallow pond near the Benares Hindu University grounds. When not producing hormogones this consisted of a mass of unbranched parallel filaments, straight or slightly curved, but not agglutinated. The colour of such strata ranged from pale brownish-green to blue-green or green, whilst older strata with abundant hormogone-formation were brownish- or greyish-yellow.

The blue-green trichomes, which are markedly constricted at the joints and exhibit fairly distinct septa, are provided with a thick mucilage-sheath. The latter is at first homogeneous (Fig. 5, A, s.), but in the older filaments

becomes differentiated into two parts. The outer sheath gradually becomes diffuent, ultimately leaving only the inner one surrounding the trichome (Fig. 5, B, *o*, *i*). The inner sheath is of roughly uniform thickness ( $1\ \mu$ ), while the outer one does not exceed  $1.75\ \mu$ . The outer sheath is soft and colourless, while the inner one possesses some degree of firmness, generally retaining its cylindrical shape when hormogones have escaped from it (Fig. 5, L and M; Fig. 6, I and J), and also occasionally at points where a number of the cells of the trichome have died (Fig. 5, G and I). The empty sheaths persist for some time after the emergence of hormogones, but ultimately become diffuent, and are dissolved. The sheaths are pectic in nature (cf. Lemaire (19) and Geitler (16)), as shown by the stains they take up. The inner sheath always assumes a slightly deeper shade than the outer.

The cells are cylindrical and generally longer than broad ( $3.1-5.2\ \mu$  broad and  $6.3-21.0\ \mu$  long), rarely quadratic in optical section. The terminal cells (Fig. 5, G), or in young trichomes, rarely also one or two of the subterminal cells (Fig. 5, H), generally taper towards the rounded apex. The cells have coarse granular contents, the granules sometimes being larger in escaped hormogones than in older trichomes. The cell contents show the same reactions as in the forms previously discussed. With stains such as aqueous methylene blue, safranin, Heidenhain's haematoxylin, &c., the central portion of the protoplast becomes deeply stained (cf. Geitler (16) and Olive (22) (Fig. 5, E and F, *c*). With chlor-zinc iodine the contents contract. Small irregular vacuoles are found in many of the cells (Fig. 5, A, B, and E, *v*), and in some they develop to such an extent that the protoplast is practically obliterated and ultimately dies. Gaps due to such dying away of cells occur here and there in the filaments. When they are short the inner sheath often becomes bent or distorted at such points (Fig. 6, B); around long gaps it usually contracts to a narrow string (Fig. 6, E), which often breaks across, leaving two filaments bearing at their ends the shrivelled remains of the inner sheath (Fig. 5, I; Fig. 6, D and J). A filament thus generally breaks into a number of pieces containing non-vacuolated cells, due to the death of cells along certain stretches. In some cases the fragments are very short, sometimes consisting of only two cells (Fig. 5, J).

The heterocysts are intercalary or terminal, and are considerably wider than the vegetative cells, so that the sheath is often bulged out opposite the points where they occur. The intercalary heterocysts are generally single (Fig. 5, A, C, and D), rarely in pairs, and are placed at more or less regular intervals throughout the length of the filament. Terminal heterocysts (Fig. 6, B-E) arise at the ends of the fragments of a trichome, produced in the way just described, and also adjacent to biconcave cells (Fig. 6, A) (cf. below). The intercalary heterocysts are always ellipsoidal

(Fig. 5, A, C, and D), whilst the terminal ones are ellipsoidal (Fig. 6, A-C),

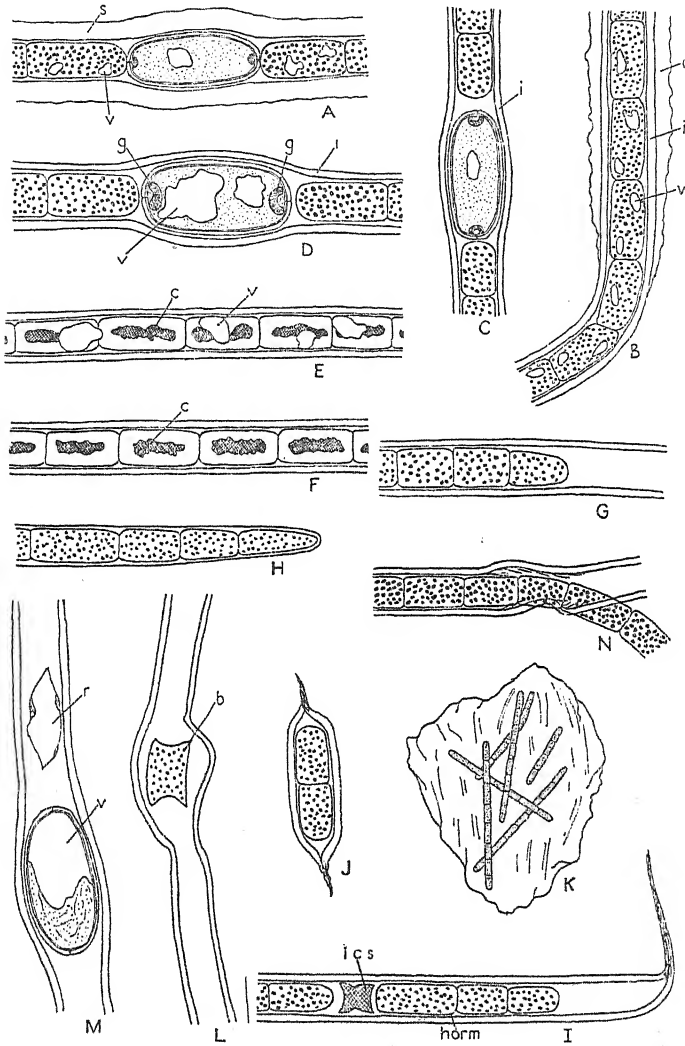


FIG. 5. *Aulosira prolifica* sp. nov. A, filament with homogeneous sheath; B, filament with sheath differentiated into two regions; C and D, filaments with inner sheath only; E and F, filaments stained with methylene blue, showing the central body; G, terminal portion of an old, and H, of a young trichome; I, trichome with a pad of intercellular substance; J, small fragment of a filament; K, mucilage formed from the sheaths intermingled with escaped hormogones; L, empty sheath with biconcave cell left behind on the escape of hormogones; M, empty sheath with old heterocyst, showing an irregular rupture for the emergence of a hormogone; N, escape of a hormogone by longitudinal splitting of the sheath. *b.*, biconcave cell; *c.*, central portion of the protoplast; *g.*, granule; *horm.*, hormogone; *i.*, inner sheath; *i.c.s.*, pad of intercellular substance; *o.*, outer sheath; *r.*, rupture in the sheath; *s.*, sheath; *v.*, vacuole. A-J and L-N  $\times 1,475$ ; K  $\times 320$ .

oval 6, D) or sub-conical (Fig. 6, J); they are always longer than broad ( $4.2-8.4 \mu$  broad and  $6.4-23.5 \mu$  long). The contents are finely granular,

and show the same behaviour towards stains as do those of the vegetative cells. The outer wall is uniformly thick all the way round, while the inner cellulose layer is very thin and rather indistinct, except around the terminal pores where it is slightly thickened. The outer wall did not show any of the reactions for pectic substances given by Tunmann (25). The terminal pores are usually very narrow and indistinct, but in rare cases they were wider, and exhibited a distinct communication between a young heterocyst and the adjoining vegetative cell (Fig. 6, B). As the heterocyst matures, this connexion disappears, and there is usually a small space between it and the vegetative cell (Fig. 5, C and D; Fig. 6, A). As a general rule a granule lies opposite each pore, and these granules appear to increase in size in the older heterocysts, sometimes being very large (Fig. 5, D, *g.*). They may be plano-convex (Fig. 5, A; Fig. 6, E), biconvex (Fig. 6, A and I), kidney-shaped or semicircular (Fig. 5, D, *g.*), with the convex side away from the pore. The granules, like those of the vegetative cells, take up a dark colour with Ehrlich's haematoxylin. The older heterocysts contain one or two large vacuoles (Fig. 5, D, *v.*), and in later stages there is very little cytoplasm, the whole cavity being practically occupied by one large vacuole (Fig. 5, M; Fig. 6, I, *v.*). The outer wall of the heterocyst is very persistent, the heterocysts or their remains being recognizable at regular intervals within otherwise empty sheaths (Fig. 5, M; Fig. 6, I and J).

In the formation of a heterocyst the adjacent cell or cells round off on the side towards the heterocystous cell (Fig. 6, F, *h.c.*), so that the latter is distinctly marked out. Later the end-walls of the heterocystous cell also round off, and at the same time the granules in the protoplast become smaller (Fig. 6, G and H, *h.c.*). The heterocyst then enlarges to its full size, while the two walls and the pores are differentiated. If a terminal heterocyst dies, the adjoining vegetative cell usually develops into a heterocyst (Fig. 6 D), so that separated portions of a trichome nearly always possess heterocysts at their free ends. The intercalary heterocysts are formed late as they are wanting in young sheathed trichomes of some length. This fact, combined with the ultimate severance of the connexion between older heterocysts and the adjoining vegetative cells, signifies that the formation of heterocysts conditions a breaking up of the trichomes into hormogones, and that intercalary heterocysts probably only arise either a little before or during the formation of the latter.

Spore-formation has not been observed, and the only method of multiplication of this alga in the present material is by means of hormogones, similar to those found by the writer (1) in *Scytonema Malaviyaensis*. These hormogones may consist of many cells or only of one (Fig. 6, C, *horm.*), two (Fig. 6, A, *horm.*), or three (Fig. 5, I, *horm.*). Such hormogones, as already described, often arise as a result of the dying of series of cells at certain points in the trichomes (Fig. 6, B, C, and E). They are also



occasionally formed by the secretion of dark green intercellular substance between two cells, such secretions often taking the form of a thick biconcave

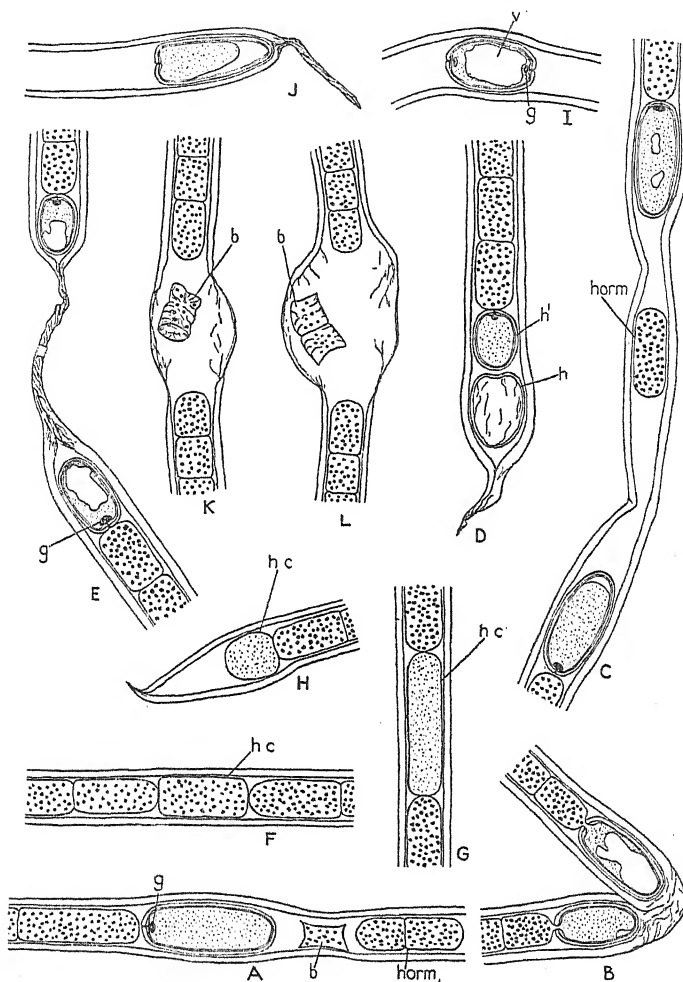


FIG. 6. *Aulosira prolifica* sp. nov. A, trichome interrupted by a biconcave cell with a terminal heterocyst at the end of one of the fragments; B-E, filaments showing the formation of heterocysts at the ends of the fragments produced by the dying of cells; F, G, and H, development of heterocysts; I and J, empty sheaths with persisting heterocysts; K and L, filaments breaking adjacent to biconcave cells where the sheath becomes dilated and more or less diffuent. *b*, biconcave cell; *g*, granule; *h*, terminal dead heterocyst; *h'*, second terminal heterocyst; *h.c.*, heterocystous cell; *horm.*, hormone; *v.*, vacuole. All  $\times 1,475$ .

pad (Fig. 5, I, *i.c.s.*), similar to those met with in many filamentous Cyanophyceae. A third method of hormone-formation is initiated by the development of intercalary heterocysts, and has already been referred to above. They are also formed by the production of single or paired biconcave cells with granular contents, such as were described by Ghose (17) in

*Aulosira fertilissima*, and have been commonly recorded in Cyanophyceae. At the points where such biconcave cells are formed, the sheath sometimes becomes dilated and more or less diffuent (Fig. 6, K and L). Later the filament breaks into two portions, and the biconcave cell, which has generally shrivelled owing to the disorganization of its contents, is thrown out. In other cases, however, the sheath remains firm (Fig. 6, A) and the biconcave cells are left behind in the empty sheath as the hormogones escape (Fig. 5, L, *b.*), although they ultimately get dissolved. The escape of the hormogones sometimes takes place through the open end of the sheath, but often by a lateral rupture of the sheath (Fig. 5, M, *r.*, and N), the heterocysts or biconcave cells, as the case may be, remaining behind, and persisting for some time within the empty sheath (Fig. 5, L, *b.*, M, *h.*; Fig. 6, I and J, *h.*). The bulk of the material consisted of loosely entangled filaments in which the trichomes were either entire or broken up into hormogones and enveloped only by the inner sheath; at other points, however, there was merely an irregular mass of empty sheaths or a mass of mucilage formed from these sheaths intermingled with escaped hormogones (Fig. 5, K).

Perennation is accomplished either by entire filaments or by fragments (sometimes only two-celled) of the filaments, or by hormogones remaining dormant inside the persistent sheaths. On the re-occurrence of favourable conditions the hormogones slowly emerge either from the open ends of the sheaths or by longitudinal irregular splitting of the latter, which are now fragile, and often exhibit a rather distorted form at these places (Fig. 5, M, *r.*, and N). The hormogones then lie intermingled with empty sheaths, which ultimately become mucilaginous and get dissolved (Fig. 5, K). Later, the hormogones secrete new sheaths and develop into the young filaments (Fig. 5, H).

The alga just described must be referred to the genus *Aulosira* in view of its possession of unbranched filaments without differentiation between base and apex, of intercalary heterocysts, and of a thick firm sheath. It differs, however, from all previously described species in (*a*) the possession of a double sheath, (*b*) the enlargement of the latter opposite the heterocysts which are considerably wider than the vegetable cells, (*c*) the exceptionally great length of the heterocysts and the presence of granular contents, even in old ones, and (*d*) the formation of terminal heterocysts at the ends of the fragments of a trichome separated by the formation of biconcave cells or by the death of intervening vegetative cells. It is also possible that the absence of spores is characteristic of this species which reproduces so abundantly by vegetative means. It may suitably be called *Aulosira prolifica* sp. nov.

4. *On the False Branching of a Species of Aulosira* (*A. pseudoramosa* sp. nov.)

The peculiar blue-green alga described below formed a flat compact bluish-green stratum with an uneven surface showing rounded prominences among mosses and liverworts on the wall of a house near the famous temple of Shri Vishvanâtha at Benares; it was collected in August, 1931.

In the ordinary vegetative state the stratum consists of unbranched filaments which are  $9.5-14.7\ \mu$  broad and up to 2 mm. long, and are generally irregularly bent and densely entangled with each other. The blue-green trichomes only occasionally show constrictions at the joints. The sheath is thick, hyaline, and stratified with parallel strata (Fig. 7, A, s.). The outer surface is somewhat uneven, while the inner surface is quite smooth except where the trichomes are constricted. At these points the sheath shows slight ring-like projections opposite the septa (Fig. 7, B, s.). The sheath is quite firm, since it retains its cylindrical form after parts of the trichomes have perished (Fig. 7, B and G), or after hormogones have escaped (Fig. 7, K). The thickness of the sheath varies between  $0.75$  and  $2.6\ \mu$ , according to the age of the filament, but in mature threads it is usually  $2.1\ \mu$ . In young filaments the sheath is closely adpressed to the trichome, but in mature ones the two are often separated by a narrow space. In older filaments the sheath in most cases gradually acquires a yellow colour, and ultimately becomes deep yellow or golden yellow. At the same time it becomes thinner, hard, and brittle. Even the occasional hyaline sheaths of mature filaments are more or less brittle, as they become irregularly broken after teasing, but the coloured sheath is always very brittle, rupturing very readily (Fig. 7, c), and even breaking of itself under natural conditions, as will be described later. The hyaline sheath is readily stained by the usual pectic stains, but as its colour changes it gradually loses the capacity to take up stains, and the deep yellow or golden yellow sheaths take practically no stain.

The cells are generally cylindrical, rarely barrel-shaped, usually slightly longer than broad, but sometimes as much as twice as long as broad; in rare cases they are shorter than broad. They are  $6.3-10.5\ \mu$  in diameter. The cell-contents are blue-green and finely granular, and react towards stains and reagents like those of the other forms previously discussed.

In old trichomes occasional vegetative cells, occurring either singly or in short or long chains, show a paling of the contents; ultimately these cells die and disappear, so that gaps are formed in the filaments. In other cases such cells persist, their contents becoming yellowish-brown or brown, and the granules usually less distinct, and eventually disappearing. These cells separate from the other living cells and, when single, appear as contracted (plano-convex, biconvex, concavo-convex, &c.) structures (Fig. 7, D,

E and I, *d*), while, when a number lie together, the whole shrinks (Fig. 7, F, *c.d.*), and becomes distorted in various ways by the growth of the adjacent living cells (Fig. 7, G and H; Fig. 8, A, *c.d.*). These dead cells take up the same stains as the living ones. Dark green biconcave discs of intercellular substance are quite commonly secreted between adjacent cells (Fig. 7, B, E, I, and J, *i.c.s.*), and are sometimes formed quite early in young trichomes and germinating hormogones. Ultimately they become brown, and stain like the living cells, being coloured very deeply by aqueous methylene blue. In some cases the cells adjacent to these intercellular discs degenerate one after the other (Fig. 7, I, *d.*). The trichomes of older filaments are thus broken into separate portions by the dying of intermediate cells, or by the formation of intercellular substance.

Heterocysts are absent from young trichomes, and are formed rather late, and only in small numbers in the mature ones. They are rarely found in trichomes with a hyaline sheath (Fig. 7, A), and are even lacking in a considerable number of those with coloured sheaths. The heterocysts are for the most part intercalary (Fig. 7, A and B), and usually occur singly, though in a few cases in pairs. Terminal heterocysts (Fig. 7, B, *h.*) are formed from the end cells of segments of a trichome separated by intercellular substance. The heterocysts are cylindrical, with rounded ends or ellipsoidal. They are generally of the same width as the vegetative cells, and are commonly slightly longer than broad, rarely twice as long as broad ( $6.3\text{--}10.5\ \mu$  broad and  $6.3\text{--}18.9\ \mu$  long). They have fine granular bluish-green contents which stain like those of the vegetative cells. The outer wall of the heterocyst is slightly thicker than the inner. The pores are not clearly visible, since there is no special thickening around them, but a granule is located opposite each. Mature heterocysts are generally isolated from the adjoining vegetative cells, so that the development of a heterocyst likewise causes a trichome to fragment. Sometimes the heterocysts become isolated by the dying of the cells on either side (Fig. 7, B). In old heterocysts the contents degenerate, and the heterocysts often become compressed (Fig. 8, C). In this condition they may be difficult to distinguish from dead cells and pads of intercellular substance, until treated with chlor-zinc-iodide, when the inner wall gives the usual deep violet reaction.

Spore-formation has not been observed, and multiplication in the present material is effected solely by hormogones, formed in the way above described. The hormogones may consist of only one, two, or three cells (Fig. 7, D and E, *horm.*) or may attain some length. By the time hormogone-formation takes place the sheath is generally coloured deep yellow or golden yellow, and within it hormogones can pass through a dormant period, their cells sometimes assuming a rounded or barrel-shaped form (Fig. 8, G, *horm.*). Owing to the brittle nature of the sheath, however, the filament may break into pieces, each enclosing one or more hormogones.

When conditions become favourable for growth, the hormogone secretes a new hyaline sheath, which is very thin and closely adpressed (Fig. 7, F, G, and I; Fig. 8, B, D, and E, *horm.*). It appears that the hormo-

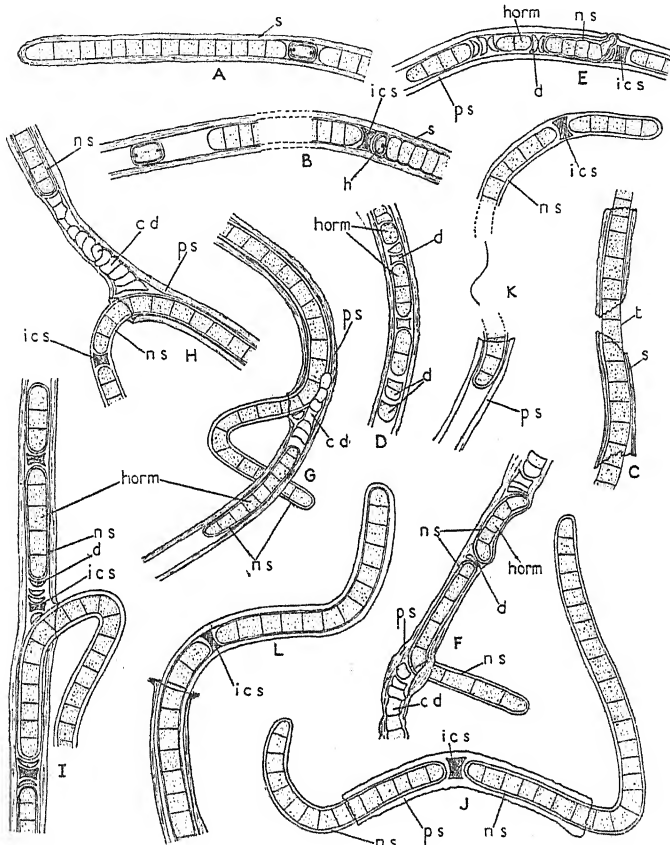


FIG 7. *Anulosira pseudoramosa* sp. nov. A and B, portions of filaments; C, filament showing rupture of the brittle sheath; D and E, breaking up of trichomes into hormogones; F-L, germination and emergence of hormogones after secretion of a new sheath. *c.d.*, chain of dead cells; *d.*, dead cell; *h.*, terminal heterocyst; *horm.*, hormogone; *i.c.s.*, intercellular substance; *n.s.*, new sheath of hormogone; *p.s.*, sheath of parent filament; *s.*, sheath; *t.*, trichome. All  $\times 320$ .

gones do not all start to grow at the same time (Fig. 7, E). The cells elongate, assume a cylindrical form, and commence to divide. When there is plenty of room inside the old sheath, the hormogone may grow to a considerable length before one of its ends penetrates the latter. The new sheath of the hormogone thickens, and becomes stratified like that of the parent filament, which by this time is often very thin and brittle; the combined thickness of the two sheaths is about  $3.1 \mu$ . By staining with 1 per cent. aqueous methylene blue the two sheaths can be distinguished (Fig. 8, J) by the deep coloration of the inner one, while the outer remains

unstained. The mode of emergence of the germinating hormogones from the old sheath varies. When they are situated near the open end of the old sheath they grow out direct (Fig. 7, L and K; Fig. 8, D), whilst when there is no such opening at hand they sometimes elongate alongside the adjoining rows of dead cells (Fig. 8, A).

When there is no other room for growth, one end of the hormogone breaks through the sheath of the parent filament (Fig. 7, E and G). Such germinating hormogones, enclosed in their own sheaths, are found emerging adjacent to dead cells (Fig. 7, F-I; Fig. 8, F, H, and I), heterocysts (Fig. 8, C) or discs of intercellular substance (Fig. 8, B, E, and G). When a hormogone penetrates the old sheath in the vicinity of a heterocyst, an appearance very similar to that of a branching *Tolypothrix* is obtained (Fig. 8, C and F). On the other hand, when adjacent ends of germinating hormogones grow out together, an appearance resembling that of the geminate branching of a *Scytonema* is realized (Fig. 8, H), especially in the rare cases in which the dead cells or intercellular discs separating the hormogones have already disintegrated (Fig. 8, I). Without adequate knowledge of the development of the alga, as here described, one might readily regard it as a branching form.

When the hormogones are short, they usually escape wholly from the coloured sheaths of the parent (Fig. 7, J and K; Fig. 8, C, D, and H), so that when a particular trichome is divided up into short hormogones only its sheath may be left altogether empty. Empty sheaths have been commonly observed, though always broken irregularly on account of their brittle nature. Escaped hormogones of this kind are more or less straight, and remain distinct from one another for some time. As they lengthen they become entangled, and the sheaths gradually change colour. Long hormogones or short ones, which have grown to a considerable length owing to the available space within the parent filament, are much delayed in their emergence. As a consequence such hormogones enveloped in their own sheath may, while some part of them is still encased within that of the parent filament, become divided up into fresh hormogones as a result of the formation of biconcave discs (Fig. 7, H, *z.c.s.*); moreover, their sheath may turn yellow before they are set free. In still other cases, which were rarely observed, hormogones which had grown to a considerable length, and were for the most part or entirely enclosed within the sheath of the parent filament, failed to emerge, and remained permanently within the latter. When the sheaths of such hormogones turn yellow, they are difficult to distinguish from the outer sheath of the parent, which is now very thin and papery. Their trichomes may in their turn become divided into hormogones, which in due course again germinate *in situ*. The old sheath of the parent is, however, ultimately thrown off, either throughout its whole length, or only at certain points; in the latter case remnants of it

are sometimes distinctly visible (Fig. 8, K, *r.*). In one exceptional case a sheathed hormogone had a considerable portion of its body inside the parent-filament, whilst the free part outside had grown to a great length ;

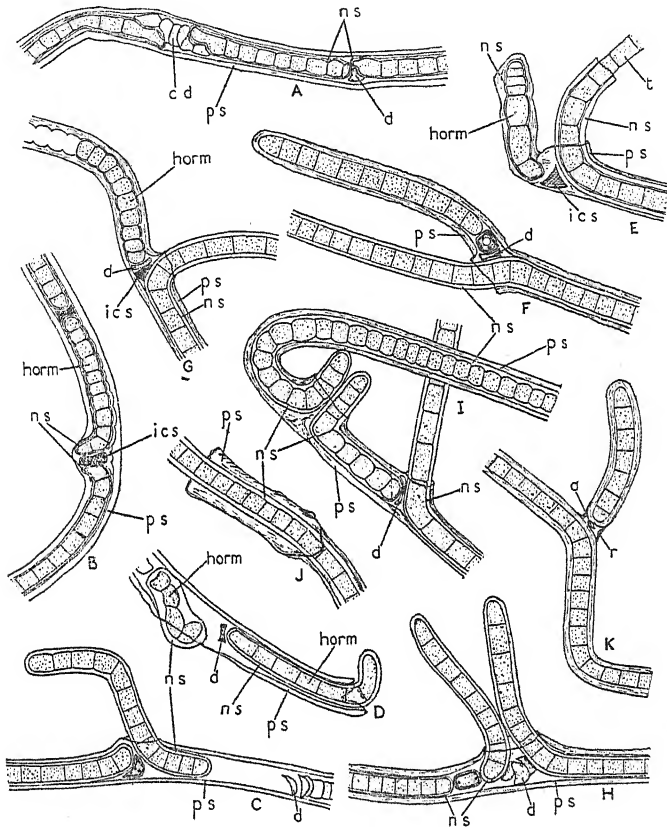


FIG. 8. *Aulosira pseudoramosa* sp. nov. A, germination and elongation of hormogones within parent-sheath ; B, two germinating hormogones ; C and D, emergence of short germinating hormogones ; E-G, emergence of long germinating hormogones, most of which are still enclosed within the parent-sheath ; H and I, two germinating hormogones emerging side by side ; J, filament with the outer sheath teased out to show the new sheath enclosing a long hormogone ; K, two hormogones, enclosed within thick coloured sheaths, joined together by a small remnant of the parent-sheath. *c.d.*, chain of dead cells ; *d.*, dead cell ; *horm.*, hormogone ; *i.c.s.*, intercellular substance ; *n.s.*, sheath of hormogone ; *p.s.*, sheath of parent-filament ; *r.*, remnant of parent sheath ; *t.*, trichome. All  $\times 320$ .

the enclosed portion and the lower part of the free one had a deep yellow sheath, while the terminal part of the free portion which was still elongating had a hyaline sheath.

In view of the marked resemblance of this form to one of the Scytomonataceae, when abundant germination of hormogones is taking place, it may be well to mention that in the false branching of the family just named there is no envelopment of the branch-trichome by a complete new sheath:

The branch-trichome secretes a new sheath of its own which can be traced back for a short distance into the main filament, but does not extend for any considerable distance backwards, terminating blindly. This has been established by examining a number of Scytonemataceae, and the writer hopes to deal with such false branching in greater detail in a later communication. Ghose (17) has described the occurrence of rare branches in *Aulosira fertilissima*, but his data do not enable one to determine their exact nature.

Since the branching of the vegetative filaments has been shown to be solely due to germination of hormogones *in situ*, the absence of differentiation between base and apex, the presence of intercalary heterocysts and the thick firm sheath show that we are dealing with a species of *Aulosira*. There is some resemblance in the possession of a thick coloured sheath to *A. fertilissima* Ghose and in the cylindrical cells and heterocysts to *A. implexa* Born. et Flah. ; but it differs from both in the irregularly bent and densely entangled filaments, and from the former in the cylindrical heterocysts with rounded ends, while the latter has a colourless sheath. The alga under discussion is also peculiar in its habitat (on walls), in its false branching, and in the absence of spores. Since the second of these features is the most distinctive, it may be named *Aulosira pseudoramosa* sp. nov.

In conclusion, I have pleasure in expressing my gratitude to Professor F. E. Fritsch for his guidance and criticism throughout this work. I am also indebted to Dr. N. Carter and Miss F. Rich for many useful suggestions and advice.

*April, 1932.*

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# The Cambium and its Derivative Tissues.

## VII. Problems in Identifying the Wood of Mesozoic Coniferae.

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With Plates III and IV.

### INTRODUCTION.

MUCH emphasis has been placed upon the occurrence of supposedly intermediate or transitional types of structure in the wood of Mesozoic Coniferae. Although most paleobotanists agree that such genera as *Protopiceoxylon*, *Protocedroxylon*, *Planoxylon*, *Araucariopitys*, &c. are generalized or transitional forms, striking differences of opinion have arisen concerning their classification and phylogenetic significance. One group of investigators considers them to be forerunners of the Pinaceae<sup>1</sup> (*Protopinaceae*) which exhibit evidences of an Araucarian or Cordaitean ancestry, whereas another group of workers asserts that they are primitive Araucarian conifers (*Araucariopityeae*) which retain vestiges of a Pinaceous ancestry.

The principal argument for regarding such conifers as generalized or transitional forms is the occurrence of so-called Araucarian pitting in combination with structures that are considered to be characteristic of the Pinaceae. The chief argument for segregating them into new hypothetical genera and subfamilies is the tacit assumption that similar combinations of structural characters do not occur in living representatives of the Coniferae.

The writer does not propose to enter the controversy concerning the relative antiquity of the Pinaceae and Araucariaceae, but to raise the question whether these putative transitional Mesozoic forms fall within the range of structural variability of living coniferous genera.

### TRACHEARY PITTING.

Before attempting to discuss this question, it is essential to determine first what is meant by 'typical Araucarian' pitting, as contrasted with the

<sup>1</sup> Pilger's (15) nomenclature for the Coniferae is used throughout this paper.

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'ordinary Coniferous' type. There appears to have been a general consensus of opinion among paleobotanists that in the former type the bordered pits are contiguous and flattened and, when in more than one vertical series, typically 'alternate'; whereas in the latter type the bordered pits tend to be more or less widely spaced and, when in more than one vertical row, characteristically 'opposite'.

The tracheary pitting of the transitional Mesozoic Conifers is not typically Araucarian but rather a mixture of putative Pinaceous and Araucarian types. Thus, in *Cedroxylon transiens* Gothan, the bordered pits may be spaced or contiguous, opposite or alternate, or arranged in stellate clusters. According to Seward (18), 'the Abietineous features predominate over the Araucarian, the latter being limited to the local occurrence of polygonal and alternate bordered pits.' Similar patches of alternating pits occur in *Protopiceoxylon extinctum* Gothan. In *Araucariopitys Americana* Jeffrey, the pits are mostly uniseriate and either widely spaced or contiguous; rarely biseriate, and then either opposite or alternate. They are stated to be contiguous and alternate in the spring-wood of *Thylloxylen irregularis* Gothan, and *Planoxylen Hectori* Stopes, but to be more or less widely spaced in the summer-wood. In *Metacupressinoxylon cedroides* (Holden) Torrey, they are described as uniseriate, spaced, or biseriate alternating.

The bordered pits of these plants are considerably larger than those which occur in living Araucarians, at least in comparable material of mature stem-wood, and certain of them possessed a well-developed torus. These are Pinaceous rather than Araucarian attributes. Furthermore, the bordered pits, when alternating, commonly tend to be less closely compressed than in typical Araucarian pitting.

It should be emphasized, in this connexion, however, that the distinctions between the two contrasted types of tracheary pitting are not as infallible diagnostic criteria as has commonly been hypothesized. They cannot be relied upon in dealing with the zone of transitional tracheides—i.e., metaxylem and first formed secondary tracheides—since mixed types of pitting occur in such regions and since, as the writer (4) has shown, the primary wood of the Coniferae is of a singularly modified and extremely specialized type. Pool (17) and others have called attention to the fact that separate and circular pits may occur in the secondary xylem of Araucarians. Contiguous and more or less flattened pits are of not infrequent occurrence in mature wood of other representatives of the Coniferae. Even the occasional occurrence of alternate pitting is not considered by Seward (18) to be an infallible criterion of Araucarian affinity. It is evident, accordingly, that the distinctions between Araucarian and Pinaceous types of tracheary pitting are quantitative rather than qualitative. In other words, the tracheary pitting of the Mesozoic fossils is to be

regarded as transitional because the proportions of contiguity, flattening, and alternation presumably are higher than in living representatives of the Pinaceae. Is such, indeed, the case?

Our systems of classifying and identifying the woods of the higher plants have developed largely through trial and error and, as the writer (1, 3) has shown, are subject to constant correction and revision. This is due to the fact that comparatively little is known concerning the limits of variability of the diagnostic criteria used in the construction of keys. The extent to which structural characters may vary in different parts of a single tree, or in trees grown under different environmental conditions, does not appear to have been appreciated fully by most of those who have concerned themselves with the identification either of commercial woods or of fossils. As will be shown in succeeding paragraphs, the variability of the tracheary pitting in the genus *Cedrus* affords an interesting verification of these criticisms.

Recently the writer had occasion to study the structure of the secondary xylem in *Cedrus Lebani* Bar., *C. Deodara* Loud., and *C. atlantica* Man. Samples of wood were secured from branches, stems, and roots of trees growing in California, Massachusetts, and Lincolnshire, England, and from the outer portions of the stems of older trees grown in their native habitats—i.e. India, Palestine, Algeria, and Morocco. Although the writer anticipated considerable variation in the tracheary pitting of *Cedrus*, he was surprised to find that all the principal types of pitting illustrated in Pl. IV, Figs. 15 to 30, occurred, not only in the genus as a whole, but within the limits of a single tree, and even of a single specimen.

The particular admixtures of different types of pitting and the proportions of contiguity, flattening, and alternation vary markedly from specimen to specimen. In wood from the branches and young stems of trees growing in parks and botanical gardens of North America and England, the tracheary pitting tends to be of the 'ordinary Coniferous' type—i.e. in each tracheide there are one or two vertical series of more or less widely spaced bordered pits, Pl. IV, Figs. 15, 17, 20, 22, and 23. Alternate pitting is absent or of sporadic occurrence, though contiguity and flattening of the Araucariopitys-type may be present. In specimens from the outer portions of the stems of what appear to have been large, old, slow-growing trees, the Pinaceous pitting may occur in combination with varying proportions of 'Araucaroid', Pl. IV, Figs. 27 and 30, and of intermediate and clustered types, Pl. IV, Figs. 16 and 21. The combinations of pitting in certain specimens closely resemble those that characterize *Cedroxylon transiens*, *Protopiceoxylon extinctum*, and other Mesozoic fossils. The most extreme cases of contiguity, flattening, and alternation encountered by the writer are in certain samples of root wood. In these, the 'Araucaroid' pitting may be greatly accentuated in the tracheides

of the spring-wood, Pl. IV, Figs. 25 and 26, and is in marked contrast to the more widely spaced pits, Pl. IV, Fig. 28, that occur in the transitional zone and summer-wood. In other words, these particular combinations of tracheary pitting approximate those that are considered to be salient diagnostic features of *Planoxylon*, *Thylloxyton*, and *Protocedroxylon araucarioides*.

It is evident, accordingly, that the tracheary pitting of the genus, *Cedrus*, is extraordinarily plastic and variable, and is of fully as 'transitional' a character as any that occurs in Mesozoic fossils. Furthermore, preliminary investigations indicate that similar admixtures of Pinaceous and Araucarian pitting occur in *Keteleeria* and other living representatives of the Pinaceae, but that in stem-wood of *Pinus*, *Picea*, *Pseudotsuga*, *Larix*, *Tsuga*, and *Abies*, contiguity, flattening, and alternation usually are of more limited and sporadic occurrence than in the root-wood of these genera or in the stem-wood of *Cedrus*.

#### SO-CALLED RIMS OR BARS OF SANIO.

The so-called rims or bars of Sanio are regarded by certain paleobotanists as the most reliable of all diagnostic criteria in distinguishing woods of Pinaceous and Araucarian affinities. The reputed absence of these structures in *Protocedroxylon*, *Protopiceoxylon*, *Planoxylon*, and other Mesozoic Coniferae is held to be a sufficient justification for classifying such genera as Araucarians rather than as Protopinaceae. Unfortunately, there is, in all probability, no more confused and contradictory chapter of paleobotanical literature than that which deals with these structures. They have not been defined accurately either from the morphological or the biochemical point of view, and structures of entirely different developmental history, as well as various artifacts, have been confused with them.

In 1924, in reviewing the work of Sifton (19) and Hale (8), the writer (2) called attention to the fact that controversies concerning the structure, distribution, and diagnostic value of the so-called rims or bars of Sanio had reached an *impasse*, which could be clarified only by detailed investigations of the cambium and its differentiating derivatives. During the last ten years, he has undertaken a number of such investigations in connexion with the work which has been described in preceding papers of this series. These investigations indicate that the developmental history of the rims or bars of Sanio, as of the torus, is morphologically and biochemically an extremely complicated story. Only the salient features need be mentioned here, as a detailed discussion will be given in a separate paper.

In the cambium of Gymnosperms and Dicotyledons the radial walls of the initials are not in close contact throughout their radial extension, but

are separated by an intercellular layer<sup>1</sup> of varying thickness. This intercellular material differs from the wall of the cambial cells in its optical and other physical properties, in its chemical solubilities and reactions with dyes, and in its physiological behaviour. It is evidently a rather plastic, colloidal substance that passes readily into a liquid or semi-liquid phase, thus facilitating those movements and adjustments—i.e. sliding growth—which are such characteristic features of the actively dividing and growing cambium. The so-called primordial pits are more or less irregularly distributed areas where the radial walls of adjacent initials are in closer contact—i.e. separated by less intercellular material. There is no evidence, however, to indicate that the 'primordial pits' are permanent structures whose position remains unaltered during the life of the cambial initials. Nor is there evidence for believing that protoplasmic connexions<sup>2</sup> occur in these areas. On the contrary, the size, number, and orientation of the primordial pit areas appear to vary,—particular areas disappearing and others being formed *de novo* during periodic movements and displacements of the intercellular substance.

In the case of the Araucarians, the so-called primordial pit areas are eliminated during the earlier stages of the development of the tracheides. Thus, the radial walls and intercellular layer are of relatively uniform thickness at the stage when the differentiation of the pit-membranes of the bordered pits is initiated. In the case of the Pinaceae, as of the Taxaceae, Podocarpaceae, Cephalotaxaceae, Taxodiaceae, and Cupressaceae, the primordial pit areas are not completely obliterated in most cases during the earlier stages of the differentiation of the tracheides, though their size and form may be modified more or less profoundly and their number augmented by subsequently formed ones. The pit-membranes of the bordered pits differentiate within these areas, so that the distribution of the bordered pits appears to be determined by the size, form, and spacing of the primary pit areas, Pl. IV, Figs. 15 to 24, 28, and 29. In the fully differentiated secondary xylem, the intercellular material that persists and is re-deposited between the primary pit areas, may be uniformly distributed, Pl. III, Fig. 14; Pl. IV, Figs. 15, 17, 20, 21, and 29, or it may be aggregated about the upper and lower margins of the primary pit areas, Pl. III, Fig. 13; Pl. IV, Figs. 16, 22, 23, and 28. It is these transverse, thicker portions of the intercellular substance that were originally designated as 'Bars of Sanio' by Gerry (5).

Where tracheary pitting is of the type illustrated in Pl. IV, Figs. 25, 26, and 30, there is obviously no room for the persistence of either 'primary pit areas' or, 'bars of Sanio'. Therefore, in searching for these structures

<sup>1</sup> The conception of the middle lamella that has arisen since the discovery of the cell-plate is not applicable to the cambium.

<sup>2</sup> Detailed investigations of 'plasmodesma' lead the writer to question whether true protoplasmic connexions occur in any of the tissues of the higher plants, with the possible exception of sieve-tubes and other highly specialized cellular systems.

in woods which have an Araucarian type of pitting, it is the unpitted or less abundantly pitted portions of the radial facets upon which attention should be focused. Furthermore, it is essential in differential staining and de-staining to guard against the production of artifacts which simulate the structures that are being searched for.

Although the writer's investigations support those of Gerry (5) and Pool (17) and justify their conclusion that 'true bars of Sanio' do not occur in the wood of the Araucariaceae, it should not be inferred from this that these structures may be used indiscriminately in the identification of fossil woods. For such an assumption presupposes that the primary pit areas and bars of Sanio are preserved in visible form under all conditions of fossilization—a clearly fallacious inference. It should be emphasized, in this connexion, that even in the wood of living representatives of the Pinaceae, Taxodiaceae, Cupressaceae, Podocarpaceae, and Taxaceae, the primary pit areas and bars of Sanio frequently may be demonstrated only after delicately controlled differential staining. The primary walls and intercellular layer vary, not only in thickness, but also in chemical composition—i.e. degree of lignification, in optical properties, and in the amount of material adsorbed during the transformation of sapwood into heartwood. Only the coarser and more refractive bars of Sanio are visible in unstained sections, and even these may be obscured in heartwood. Similarly, they may be visible in one portion of a fossilized specimen and entirely invisible in others. That the reputed absence of bars of Sanio in the Protopinaceae is due, in part, to their having been obscured during fossilization and, in part, to a failure to recognize them when visible, is indicated by the writer's investigations of Protopiceoxylon.

#### PROTOPICEOXYLON Gothan.

The genus Protopiceoxylon was instituted by Gothan (6, 7) for some Lower Cretaceous wood from King Charles Land. Three species have been described, *P. extinctum* Gothan, *P. articum* Seward, and *P. Edwardsi* Stopes. The salient diagnostic feature of the genus—and the one in which it is supposed to differ, not only from other genera of the Protopinaceae, but also from all other gymnosperms, both living and extinct—is the occurrence in the secondary xylem of 'normal' resin canals in the vertical direction only. *Pinoxylon dacotense* is excluded by both Gothan and Seward on the grounds that it is not possible to determine from Knowlton's (14) description whether the vertical canals are normal or traumatic. Recently, through the courtesy of Mr. Charles B. Read of the U.S. Geological Survey, the writer has had the privilege of examining sections of Knowlton's type specimen. The structural details, even of the tori and pit-membranes, are beautifully preserved. The vertical resin canals are of the so-called 'normal' type, and the specimen so closely resembles those



that have been classified as Protopiceoxylon that it obviously cannot be referred to a separate genus.

In Gothan's photomicrograph of a radial section of *Protopiceoxylon extinctum*, the pitting in certain of the tracheides is of the type illustrated in Pl. IV, Figs. 20-22, whereas in others it is of the type shown in Pl. IV, Fig. 30. The paired and clustered pits of the former elements appear to have been formed over primary pit areas as in Pl. IV, Figs. 20-22. Similar combinations of opposite, clustered, and alternate pitting occur in Knowlton's specimen. Bars of Sanio are clearly visible between the pairs of opposite pits in certain of the tracheides, though they have been obscured by processes of silicification throughout the summer-wood and most of the spring-wood.

Pl. III, Fig. 12, is a tangential longitudinal section of a Protopiceoxylon from the 'Tar Sands' (Lower Cretaceous) of Northern Alberta. In this tar-impregnated lignite, the tori and bars of Sanio are well preserved in certain regions of the specimens and resemble those illustrated in Pl. III, Fig. 10. This Protopiceoxylon, a log more than 30 ft. in length, was discovered by Mr. Ells, of the Canadian Department of Mines, in close association with stems of Xenoxylon and Phyllocladoxylon.

Protopiceoxylon obviously is not an Araucarian Conifer. Is it an extinct Protopinaceous genus, transitional between Cedroxylon and Piceoxylon? The only remaining argument for considering it to be such is the assumption that a similar distribution of resin canals does not occur in living representatives of the Pinaceae.

#### KETELEERIA Carr.

Comparatively little is known regarding the anatomy of this interesting representative of the Pinaceae. References concerning the structure of the xylem are few in number and extremely contradictory. Pirotta (16) states that resin canals are irregularly distributed in the secondary xylem of the root of *K. Fortunei* Carr., but that he did not succeed in finding any in the wood of the stem. Jeffrey (11) concludes that 'Keteleeria in its anatomy obviously belongs with *Abies*, although it has the habit of *Cedrus*; the wood of both vegetative and reproductive axes is entirely without resin canals'. Holden (9) likewise expresses the opinion that 'Keteleeria has the wood structure of *Abies*. Ray tracheides are entirely absent, even in such primitive regions as the first annual ring, cone-bearing branch, cone axis, and are not recalled after wounding, though there is an abundant formation of traumatic resin canals.' Hutchinson (10), who accepts Holden's conclusions, figures a transverse section of a young branch of *K. Fortunei*; there are no resin canals in the xylem. Kanehira (12), in describing the wood of *K. Davidiana* Beissn., states: 'Vertical resin ducts small, distributed evenly or in groups . . . ray tracheides present, without

spirals'. In Kanehira's (12, 13) photomicrographs of the wood of *K. Davidiana*, the resin canals are widely spaced, but are arranged in tangential series. Therefore, the objection may be raised, as was done in the case of *Pinoxylon dacotense*, that the canals are of traumatic origin.

Although Kanehira's selection of illustrative material may have been somewhat unfortunate—he figures a similar type of structure in *Picea morrisonicola* Hay.—his diagnosis of the mature stem-wood of *K. Davidiana* is accurate. As shown in Pl. III, Figs. 1 and 2, the distribution of the vertical resin canals is of the so-called 'normal' type, and is the same as in corresponding stem-wood of *Picea*, *Pseudotsuga*, *Larix*, and *Protopiceoxylon*; but in *Keteleeria Davidiana* and *Protopiceoxylon* there are no horizontal canals, except such as may occur sporadically in the immediate vicinity of certain types of injuries. In *Keteleeria*, as in *Picea*, the innermost layers of the secondary xylem may at times be entirely devoid of resin canals, or they may have only vertical canals of the aggregated or so-called traumatic type, Pl. III, Fig. 4.

It should be noted in this connexion, however, that Thomson and Sifton (20) have raised the question whether any of the resin canals in the secondary xylem of the Abietoideae are of 'normal' origin. They present considerable evidence to show that even the isolated or diffused vertical resin canals of *Picea* are induced by traumatic or other abnormal environmental stimuli. From this point of view, the various genera of the Abietoideae form a graded series of increasing (or decreasing) sensitivity to environmental stimuli. In such super-sensitive plants as *Picea*, *Pseudotsuga*, *Larix*, and *Keteleeria Davidiana*, resin canals are always present in the adult wood, owing to the cumulative overlapping effects of successive abnormal stimulations. Furthermore, Thomson's and Sifton's investigations suggest that the sensitivity of the tissues in the cambial region may vary in different parts of a tree and during different stages of its development.

In any case, it is evident that *Protopiceoxylon* falls within the range of structural variability of *Keteleeria*, not only as regards the peculiar distribution of its resin canals, but also as concerns its tracheary pitting, tori, bars of Sanio, rays, and other structural features. The specific combinations of anatomical structures that characterize such *Protopiceoxyla* as *P. extinctum*, *P. articum*, *P. Edwardsi*, and *Pinoxylon dacotense* may be duplicated in different specimens of the wood of *Keteleeria*. Furthermore, the pith of *Keteleeria* may, at times, be provided with sclerenchymatous diaphragms, Pl. III, Figs. 7 and 9, as in certain of Gothan's type-specimens of *P. extinctum*, or it may be devoid of them, as in *P. Edwardsi*. Indeed, it is a question whether the various types of *Protopiceoxylon* may not eventually be duplicated in a set of specimens secured from different parts of a single tree.

# DISCUSSION AND CONCLUSIONS.

During the last twenty-five years, at least fourteen new genera of Mesozoic Coniferae have been instituted for woods of supposedly intermediate or transitional structure. This increasing assemblage of hypothetical genera forms one of the main whirlpools in the stream of controversy concerning the relative antiquity of the Araucariaceae and Pinaceae. As noted in the introduction, the principal argument for regarding these Mesozoic Conifers as generalized or transitional forms is the occurrence of so-called Araucarian tracheary pitting in combination with structures that are not essentially Araucarian. The chief argument for segregating them into new genera and subfamilies, or families, is the tacit assumption that similar combinations of structural characters do not occur in living representatives of the Coniferae. Both these premises are unreliable, at least in so far as the genera listed in the first of the following categories is concerned.

## I.

'*Abietineous*' ray-pitting.  
Planoxylon Stopes.  
Protocedroxylon Gothan.  
Protopiceoxylon Gothan.  
Thylloxyton Gothan.  
Araucariopitys Jeffrey.  
Metacupressinoxylon Torrey.

## II.

*Non-Abietineous* ray-pitting.  
Anomaloxylon Gothan.  
Brachyoxylon Hollick & Jeffrey.  
Paracedroxylon Sinnott.  
Protobrachyoxylon Holden.  
Paracupressinoxylon Holden.  
Paraphyllocladoxylon Holden.  
Telephragmoxylon Torrey.  
Xenoxylon Gothan.

The tracheary pitting and other structural characters of Protocedroxylon, Protopiceoxylon, Thylloxyton, Planoxylon, &c., fall within the range of variability of living representatives of the Abietoideae. If these fossils are to be classified as Protopinaceae or Araucariopityeae, then so must fragments of the wood of Cedrus, Keteleeria, and other extant genera; clearly a *reductio ad absurdum*. Furthermore, preliminary investigations suggest that the tracheary pitting of most, if not all, of the genera listed in the second category of putative transitional forms falls within the range of variability of living representatives of the Podocarpaceae, Taxodiaceae, and Cupressaceae.

This paradoxical situation, and many others, have arisen owing to a dearth of extensive and reliable information concerning the limits of structural variability in living representatives of the Coniferae. They cannot be fully clarified until large collections of authentic specimens are assembled, not only from different genera, species, and geographical races, but also from different parts of the tree and from trees grown under different

environmental conditions. The information available at present is so fragmentary and unreliable that it is not possible to construct dependable keys for distinguishing even the commercial woods of many of the genera of Coniferae, to say nothing of species. Furthermore, although the normal adult stem-wood of the Araucariaceae may be readily distinguished from that of the Pinaceae, and this, in turn, from that of the remaining families of the Coniferae, there are no reliable criteria, as yet, for separating many of the Taxodiaceae, Cupressaceae, and Podocarpaceae.

In view of such facts as these, a number of pertinent questions arise concerning the wisest procedure in dealing with fossil woods. The writer is of the opinion that sufficient material from diverse sources has been examined to indicate that the Pinaceae may be distinguished by the character of their ray-pitting. It is true that the so-called Abietineous type of ray-pitting, as originally defined by Gothan, is not an infallible criterion, since superficially similar types of ray-pitting occur, at times, in certain of the Cupressaceae and other families. But, as will be shown in a separate paper, the rays of the Pinaceae are characterized by having numerous pits which communicate with the intercellular spaces. Furthermore, the pits between adjacent parenchymatous cells of the rays are of a fundamentally different type from those that occur in the Taxodiaceae and Cupressaceae.

Within the Pinaceae there are three groups of genera whose wood may be distinguished with a considerable degree of certainty. These are :

1. *Pinus*.
2. *Picea*, *Pseudotsuga*, and *Larix*.
3. *Keteleeria*, *Cedrus*, *Pseudolarix*, *Abies*, and *Tsuga*.

It may be objected that the wood of *Pseudotsuga* is characterized by having tertiary spiral thickenings in the tracheides of both spring-wood and summer-wood ; that of *Keteleeria*, by the distribution of its resin canals ; and that of *Cedrus*, by its peculiar tori ; and that these genera should be placed in separate categories. Unfortunately, tertiary spirals occur, at times, in both spring-wood and summer-wood of certain species of *Picea*. The adult wood of *Keteleeria* has been studied in one species only. Although there is some evidence to indicate that the torus, Pl. IV, Figs. 18 and 19, may eventually provide reliable criteria for differentiating the wood of *Cedrus*, *Tsuga*, and other genera of the Abietoideae, it is not possible, as yet, to state that such is actually the case. Therefore, the wisest procedure in dealing with woods of obvious Pinaceous affinities is to refer them, *for the present*, to one of the following form-genera, rather than to attempt to classify them under extant or hypothetical extinct genera.

1. *Pinuxylon*.

For woods which fall within the range of structural variability of the genus *Pinus*.

2. *Piceoxylon*.

For woods which fall within the range of structural variability of *Picea*, *Larix*, and *Pseudotsuga*.

3. *Cedroxylon*.

For woods which fall within the range of structural variability of *Keteleeria*, *Pseudolarix*, *Cedrus*, *Tsuga*, and *Abies*. (Here should be included *Planoxylon*, *Pinoxylon*, *Protopiceoxylon*, *Thylloxyton*, *Protocedroxylon* or *Metacedroxylon*, *Araucariopitys*, and *Metacupressinoxylon*.)

It should be emphasized in conclusion that, in the present status of our knowledge concerning the variability of diagnostic criteria, specific names as applied to fossil woods have no significance other than as aids in designating particular specimens. The word 'species' must be used in an entirely different sense from that in systematic botany. The failure to recognize this fact has led to much confusion and to numerous misleading generalizations.

SUMMARY.

1. The tracheary pitting of the genus *Cedrus* is plastic and variable, and of fully as intermediate or transitional a character as any that occurs in the so-called *Protopinaceae* or *Araucariopityeae*.

2. The distribution of the resin canals in the adult wood of *Keteleeria Davidiana* is of the same type as occurs in *Protopiceoxylon* and *Pinoxylon dacotense*.

3. Such hypothetical transitional genera as *Protocedroxylon*, *Protopiceoxylon*, *Planoxylon*, *Thylloxyton*, &c., fall within the range of structural variability of *Cedrus*, *Keteleeria*, and other genera of the *Pinaceae*. If they are to be classified as *Protopinaceae* or *Araucariopityeae*, then so must fragments of the wood of *Cedrus*, *Keteleeria*, and other genera of the *Pinaceae*.

4. This paradoxical situation, and many others, have arisen owing to a dearth of extensive and reliable information concerning the limits of structural variability in living representatives of the *Coniferae*. They cannot be fully clarified until large collections of authentic specimens are assembled, not only from different genera, species, and geographical races, but also from different parts of the tree and from trees grown under different environmental conditions.

The writer is indebted to Professor S. J. Record for material of *Cedrus* and *Keteleeria*.

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## EXPLANATION OF PLATES III AND IV.

Illustrating Dr. I. W. Bailey's paper on 'The Cambium and its Derivative Tissues. VII. Problems in Identifying the Wood of Mesozoic Coniferae'.

### PLATE III.

Fig. 1. *Keteleeria Davidiana*. Transverse section of mature stem-wood, showing distribution of resin canals.  $\times 20$ .

Fig. 2. *K. Davidiana*. Transverse section of mature stem-wood, showing distribution of resin canals.  $\times 20$ .

Fig. 3. *K. Davidiana*. Portion of Fig. 1, more highly magnified, showing resin canal and contrast in density between late-wood and early-wood.  $\times 120$ .

Fig. 4. *K. Davidiana*. Transverse section of young branch, showing arc of 'traumatic' resin canals.  $\times 50$ .

Fig. 5. *K. Davidiana*. Portion of Fig. 2, more highly magnified, showing resin canal and parenchyma on the outer surface of the late-wood.  $\times 120$ .

Fig. 6. *Keteleeria Davidiiana*. Radial longitudinal section of xylem, showing cyst-like appearance of resin canal.  $\times 120$ .

Fig. 7. *K. Davidiiana*. Longitudinal section through the pith of small twig, showing transverse plates of thick-walled parenchyma.  $\times 14$ .

Fig. 8. *K. Davidiiana*. Tangential longitudinal section of xylem, showing uniseriate and biseriate rays. (Compare with Fig. 11 for variations in ray structure.)  $\times 120$ .

Fig. 9. *K. Davidiiana*. Portion of Fig. 7, more highly magnified, showing two types of pith cells.  $\times 90$ .

Fig. 10. *K. Davidiiana*. Tangential longitudinal section through type of tracheary pitting illustrated in Fig. 22. The torus and thicker portions of the middle lamella are deeply stained in contrast to the lightly-coloured secondary walls.  $\times 650$ .

Fig. 11. *K. Davidiiana*. Tangential longitudinal section of xylem, showing that the cyst-like appearance of the resin canal in Fig. 6 is due to the inclusion of ray tissue. Rays small and uniseriate throughout.  $\times 120$ .

Fig. 12. *Cedroxylon (Pinoxylon) species*. Tangential longitudinal section through four bordered pits, showing four tori and three double 'Bars of Sanio'.  $\times 830$ .

Fig. 13. *K. Davidiiana*. Tangential longitudinal section through type of tracheary pitting illustrated in Fig. 23. The deeply-stained middle lamella has a beaded appearance.  $\times 500$ .

Fig. 14. *K. Davidiiana*. Tangential longitudinal section through type of tracheary pitting illustrated in Fig. 17. Between the bordered pits, the deeply-stained middle lamella is of relatively uniform thickness.  $\times 500$ .

#### PLATE IV.

Fig. 15. *Cedrus Deodara*. Radial longitudinal section of stem-wood, stained to reveal the primary pit areas, so-called bars of Sanio, pit membranes, and tori. The outlines of the bordered pits coincide with those of the pit membranes.  $\times 370$ .

Fig. 16. *Keteleeria Davidiiana*. Radial longitudinal section of stem-wood, showing outlines of clustered bordered pits in widely separated primary pit areas.  $\times 370$ .

Fig. 17. *C. Deodara*. Radial longitudinal section of the stem-wood, showing uniform thickening of middle lamella between widely-spaced primary pit areas.  $\times 370$ .

Fig. 18. *K. Davidiiana*. Portion of Fig. 16, more highly magnified, showing primary pit area, so-called bars of Sanio, outlines of bordered pits, pit membranes, and tori.  $\times 1850$ .

Fig. 19. *C. Deodara*. Portion of Fig. 15, more highly magnified, showing primary pit area, so-called bars of Sanio, pit membrane, and torus.  $\times 1850$ .

Figs. 20-24. *K. Davidiiana*. Portions of radial facets or tracheides, stained to reveal the primary pit areas, so-called bars of Sanio, pit membranes, and tori. The outlines of the superimposed bordered pits coincide with those of the pit membranes.  $\times 370$ .

Fig. 25. *Cedrus Lebanii*. Radial longitudinal section of root-wood, showing triseriate alternating arrangement of pits and entire absence of so-called rims or bars of Sanio.  $\times 400$ .

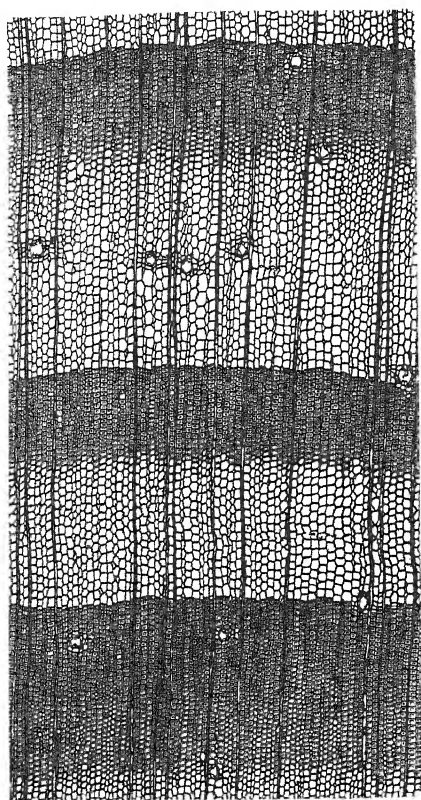
Fig. 26. *C. Lebanii*. Radial longitudinal section of root-wood, showing biseriate alternating arrangement of pits.  $\times 400$ .

Fig. 27. *Cedrus Deodara*. Radial longitudinal section of stem-wood, showing compressed uniseriate pitting.  $\times 370$ .

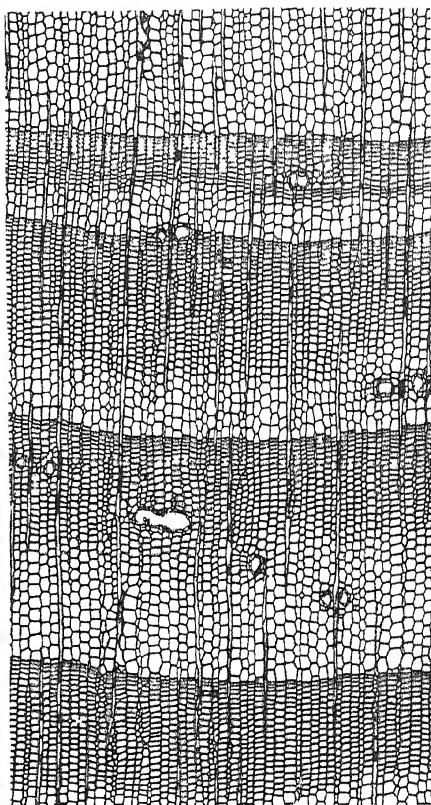
Fig. 28. *C. Lebanii*. Radial longitudinal section of root-wood, showing widely separated primary pit areas and so-called rims or bars of Sanio.  $\times 400$ .

Fig. 29. *C. Lebanii*. Radial longitudinal section of root-wood, showing clustered, compressed pits in large primary pit areas.  $\times 400$ .

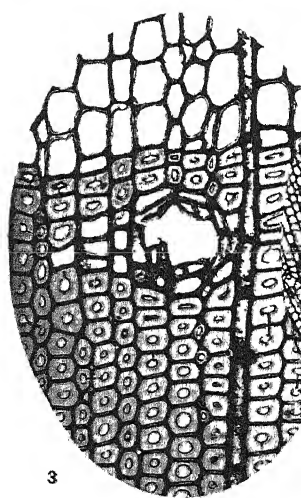
Fig. 30. *C. Deodara*. Radial longitudinal section of stem-wood, showing alternating arrangement of pits and absence of so-called rims or bars of Sanio.  $\times 370$ .



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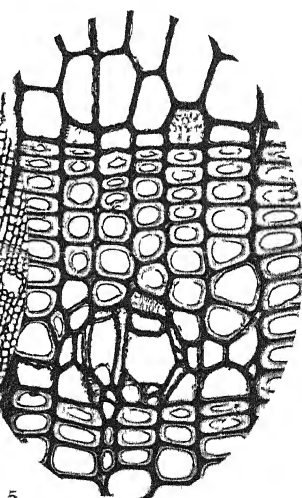
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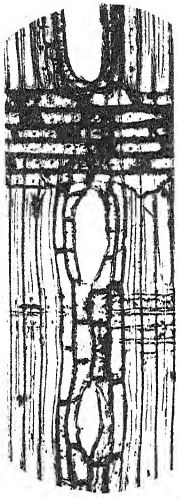


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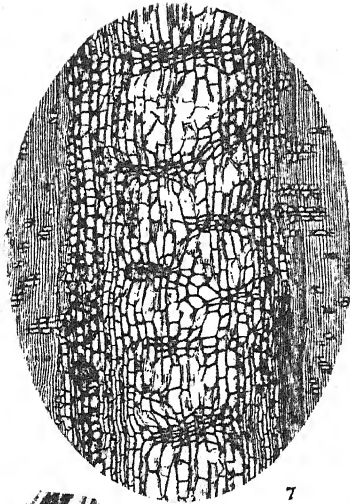


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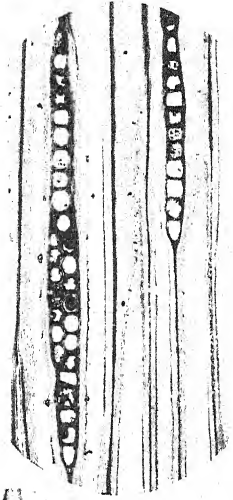




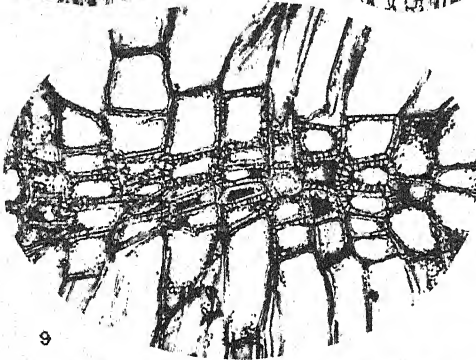
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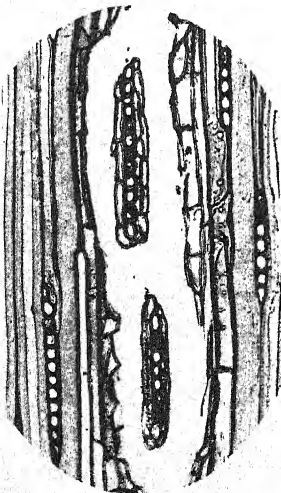
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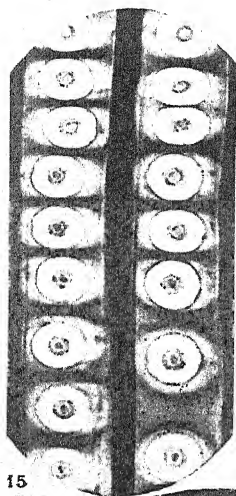
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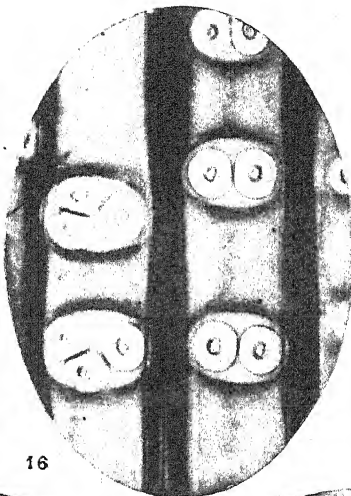
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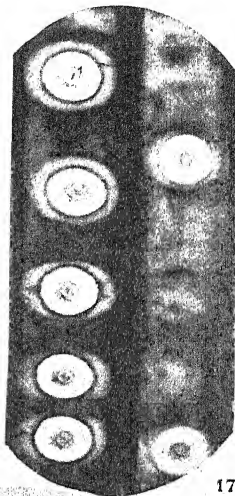
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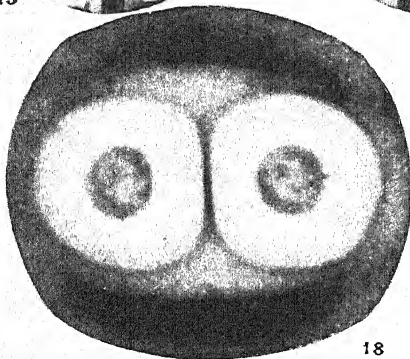
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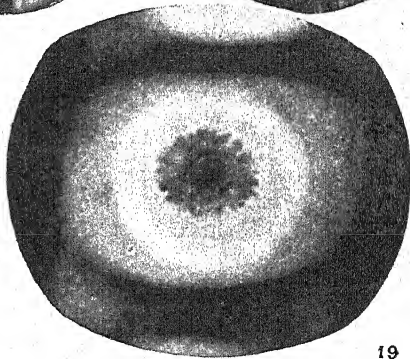
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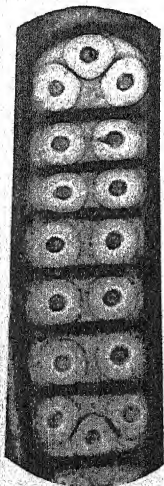
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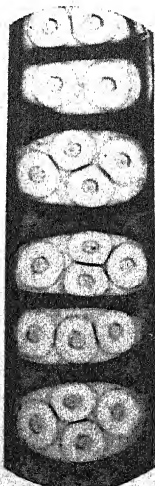
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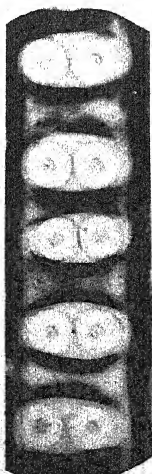
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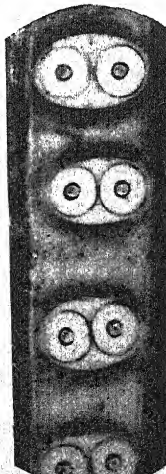
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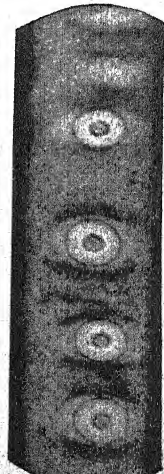
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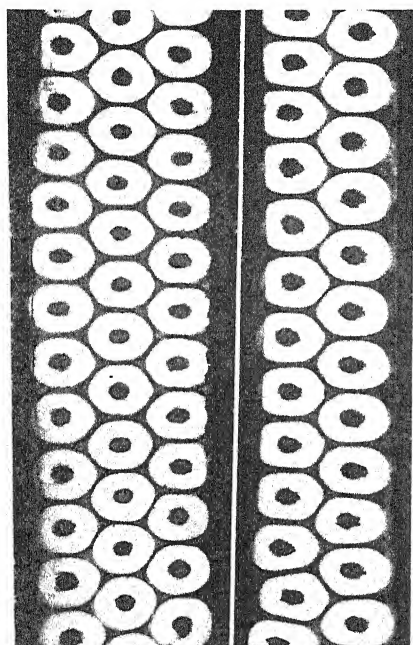
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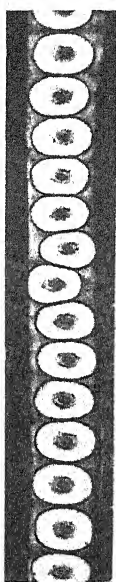


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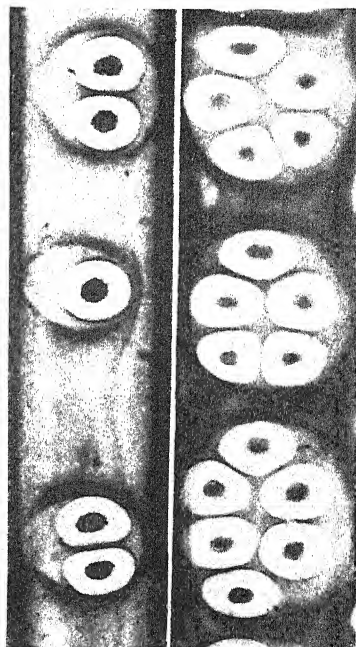


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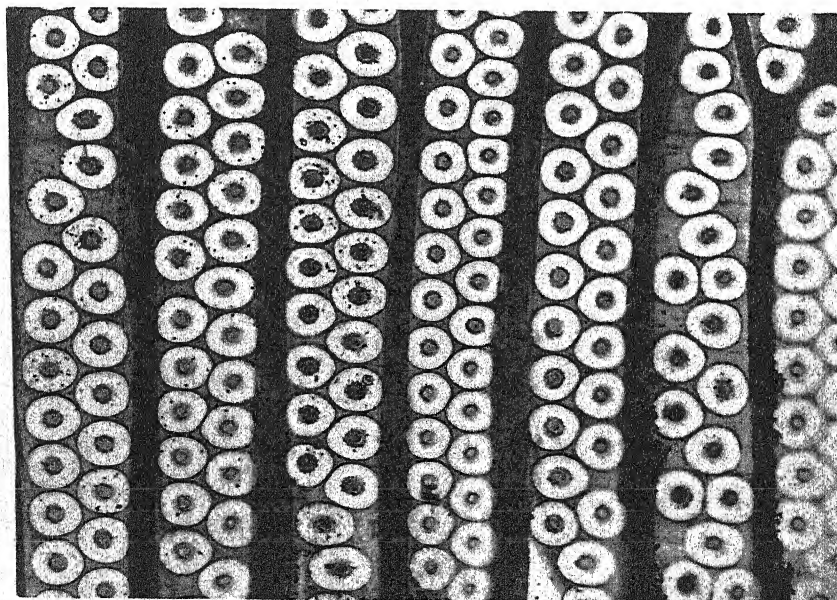


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# The Anatomical and Physiological Relation between *Hydnora Solmsiana* Dinter and its Host.

BY

A. C. LEEMANN.

(Division of Plant Industry, Pretoria.)

With Plates V and VI.

*HYDNORA Solmsiana* was first described by Dinter from specimens collected in South West Africa, where it appears to be extremely abundant, so much so that it has been used as a tanning material. In 1930 the National Herbarium in Pretoria received a specimen from the northern Transvaal,<sup>1</sup> which gave me an opportunity of examining the plant anatomically and its attachment to the host. Few investigations have been carried out in that respect on South African phanerogamic parasites, and there still remains a wide field for very interesting investigations.

Pl. V, Fig. 1 shows the root of an acacia with the parasite already well-established at E. At D, C, B, and A of the same figure we find four other specimens of different ages in various stages of association with the root.

The plant is reduced to a fleshy body devoid of roots, branches, and leaves, showing a complete degeneration in all parts. It has no typical geometrical shape, and more or less resembles a somewhat flattened root covered with warts which Dinter regards as representing undeveloped flowers. *Hydnora africana* Thunb., considered for comparison with the species with which we are dealing, is described by Marloth (10) as consisting of an 'underground angular stem, which is covered with tubercles, but produces no roots, attaching itself to the roots of the host by sending suckers into its tissue. At these spots the euphorbia root swells considerably, and as the free end usually dies, such a root does not feed its own plant any more, but serves merely as a feeding-tube of the parasite, through which the building materials which it requires are drawn from the host'. Apart from the shape of the stem, this description also applies to *H. Solmsiana*

<sup>1</sup> Sent by Mr. P. G. van der Byl, Beijeskuil, Pietpotgietersrust.

on the acacia root. Our photograph shows quite clearly the swelling of the root of the host, its dying terminal portion, and its reduction to an extended sucker of the parasite.

The longitudinal section in Pl. V, Fig. 1, shows the warty black surface and the vascular bundles as white streaks. The fleshy tissue is dark red in colour, which indicates a high tannin content. The physiological significance of the presence of these tannins is not as yet quite clear. As many of them are in the form of glucosides they have in the past been considered as reserves. The red colour indicates, however, that phlobaphenes, which are the oxidation products of many tannins are probably present. It would evidently lead us too far, were we to indulge in a discussion on the physiological value of these tannins to the parasite, yet we feel compelled to stress the point that it is a most interesting question. In this respect the view of Palladin that the tannins when hydrolysed provide acceptors and chromogenes which he considers to be involved in the respiratory process, should be considered. We must admit that the respiration of the parasite is somewhat hampered by the reduction of the surface compared with its mass and by its underground position. The supply of oxygen through the roots seems negligible. The view of Wieland that the oxygen of the respiratory process is always drawn from water, the hydrogen being bound by a hydrogen acceptor, should also receive consideration. It may be stated that I have found peroxylase along the vascular bundles of the parasite. These few remarks suffice to indicate the problem of respiration of these underground parts, and its possible relation to the presence of such an abundance of tannins.

The region where the parasite is attached to the roots of its host is shown on an enlarged scale (2x) in Pl. VI, Fig. 2. The parasite sends suckers into the tissues of the root, and the latter responds by swelling, and the production of cup-shaped outgrowths. The section shows quite clearly a strong convergence of vascular bundles of the parasite towards these suckers, whence it derives its nutrition.

An enlarged view of the cup-shaped outgrowth of the root and the sucker of the parasite, which it surrounds, is shown in Pl. VI, Fig. 3. The main body of the root is on the right. The photograph shows quite distinctly the main traces of the vascular bundles, as they bend off at nearly right angles to form the cup and to meet the sucker of the parasite. This bending of the vascular bundles is particularly well represented in the lower right-hand corner. The sucker of the parasite is easily distinguished from the strands of vascular bundles of the root. Even at this low magnification the honeycomb structure of the cap of the sucker is obvious. In our picture, however, there seems to be a special arrangement of some cells in two straight lines leading from the very tip of the cap to the white semi-circle, and we see some vascular bundles meeting these two lines. The

vascular bundles of the host stop abruptly on a semicircle beneath the cap, which is entirely free from them.

Pl. VI, Figs. 4 and 5 show more clearly that region where the vascular bundles of the parasite end, and where the general stroma or parenchyma of the parasite merges into a tissue of a new type, constituting the cap of the sucker. The stroma of the cap is composed of large, thickly walled cells, imbedded in which are a great number of giant, more or less, ovoid cells. These giant cells seem to be but loosely inserted on the underlying stroma, they are up to  $115\mu$  in length and  $75\mu$  in width. When treated with eau de Javelle these giant cells take up the most varied colours—red, green, yellow, brown, purple and orange, whereas the underlying tissue is greenish. Huge drops of fat, standing with Sudan III and alkannin, are found within the giant cells. Other conspicuous bodies they contain are not stained by Sudan III, but take a brown tint in iodine and are coloured blue by Nile blue. In a solution of 10 per cent. glucose some of the giant cells show a strong plasmolysis, others respond only slightly, and some do not react at all. In assuming that this concentration of glucose is just striking the average osmotic pressure, we would say that they possess a pressure of 12 to 13 atm. or more. Pl. VI, Fig. 5, shows one of these giant cells with a vascular bundle ending in its neighbourhood.

The mechanism of absorption of nutriment by the parasite from the host would appear to be as follows: The host brings its sap right up the cap of the sucker where it will be absorbed by the giant cells, which will transmit it to their own vascular bundles. We may, therefore, also call this zone the absorption or transmission zone. This transmission of nutritive substances from one system of vascular bundles to another, simple as it may seem, raises many puzzling questions. It reminds one distinctly of the problem involved in nectar secretion, where a certain number of living cells are involved in the absorption of cane sugar, in the transformation of this into invert sugar, and finally in the most puzzling activity of secreting this sugar externally, in spite of semipermeability. In the case of our parasite a similar thing is happening, with the only difference that it is not into the open, but into another conducting system that the nutritive substances are thrown. How this process of transmission can be accounted for by the osmotic pressure, or in spite of it, remains to be explained. It is of interest to note that the host carries its starch right up to the vicinity of the cap of the parasite, from which fact we might infer that the transmission zone is capable of making use of these starch reserves, altering the starch by enzyme action and absorbing the sugar.

It may be argued that these giant cells are haustorial cells, but of peculiar shape, and may thus have the same function, although they do not possess the capacity of elongation which gives these haustoria a resemblance to the hyphae of fungi.

# SUMMARY.

A study of the anatomical relations between *H. Solmsiana* and its host shows the vascular bundles of the host converging towards a cup-shaped outgrowth in contact with the sucker of the parasite. This sucker bears a special cap, forming a zone devoid of vascular bundles, which transmits the nutritive substances from the vascular system of the host to the vascular system of the parasite. The cap shows a great number of giant cells, which probably are the active intermediates, acting as haustorial cells between the host and the parasite.

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# EXPLANATION OF PLATES V AND VI.

Illustrating Dr. A. C. Leeman's paper on 'The Anatomical and Physiological Relation between *Hydnora Solmsiana* Dinter and its Host'.

## PLATE V.

Fig. 1. Root of an acacia with parasite well-established (E). A, B, C, D, four other specimens of different ages in various stages of association with the root.

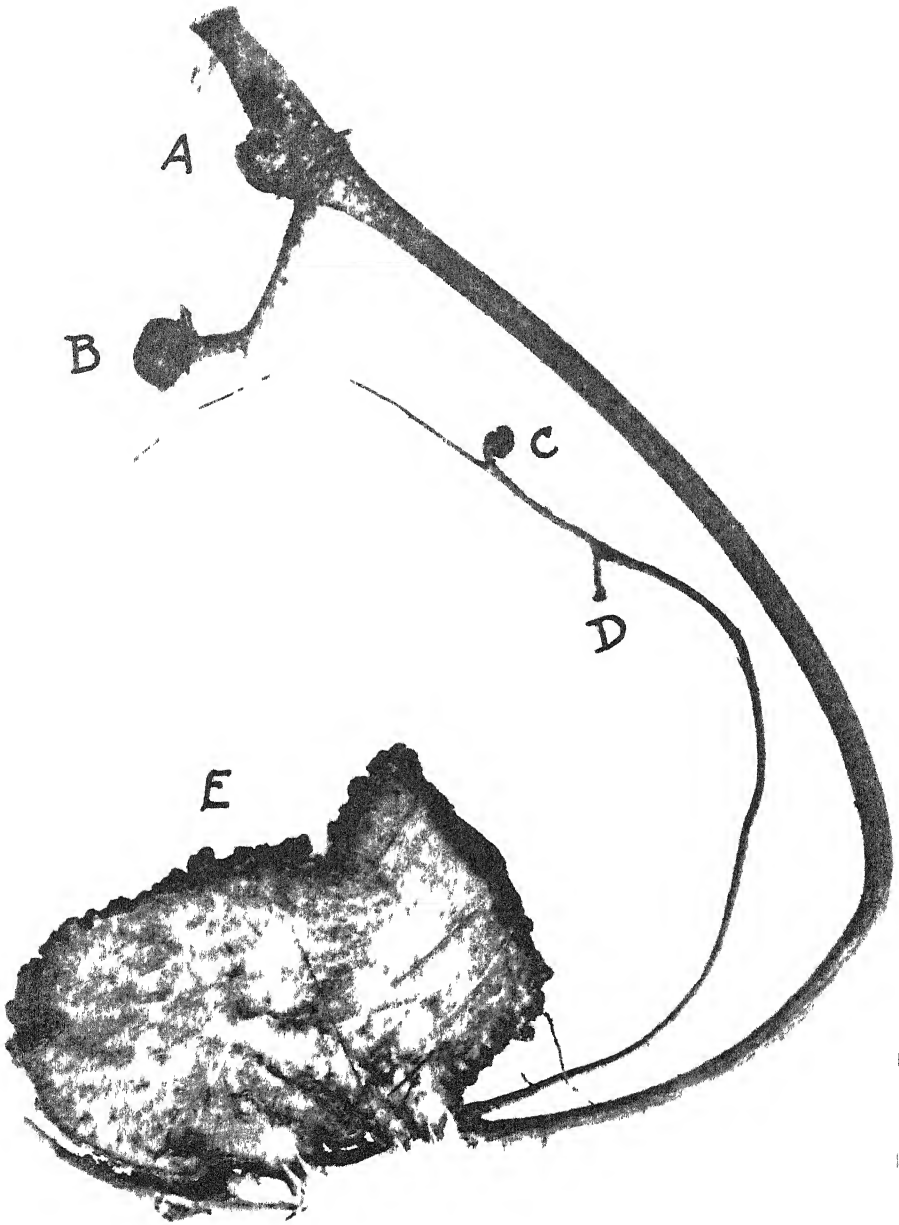
## PLATE VI.

Fig. 2. Region of attachment of parasite to root of host.

Fig. 3. Enlarged view of the cup-shaped outgrowth of the root and the sucker of the parasite.

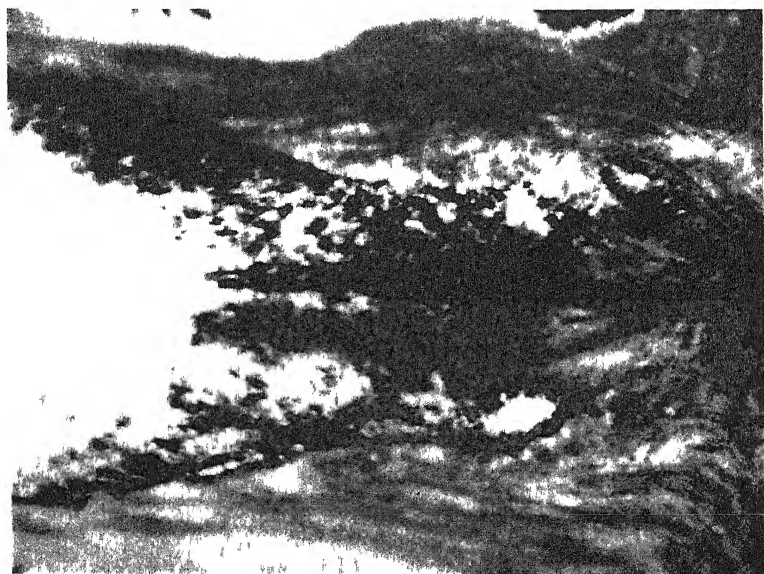
Figs. 4 and 5. Place of ending of the vascular bundles of the parasite and the merging of the general stroma of the parasite into a new tissue, constituting the cap of the sucker. Fig. 4. General view. Fig. 5. Enlargement of part of Fig. 4, showing one of the giant cells with a vascular bundle ending near it.



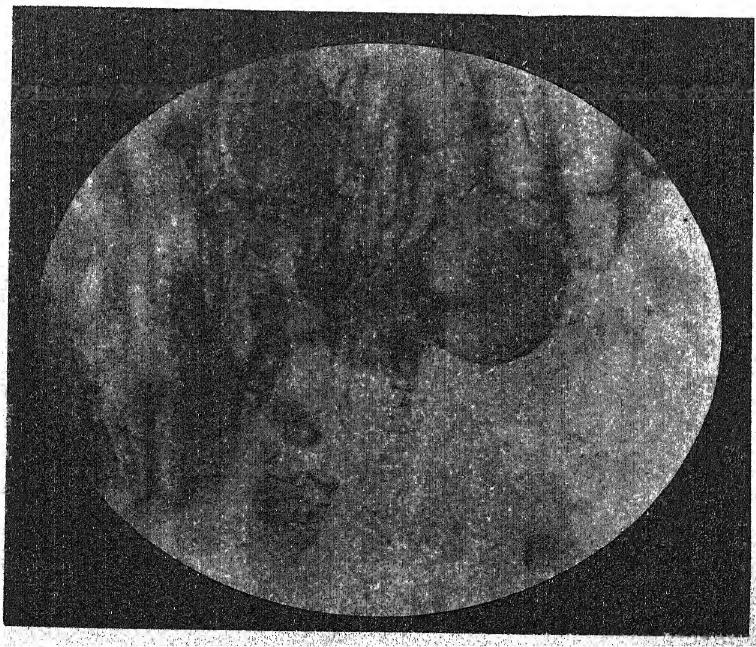
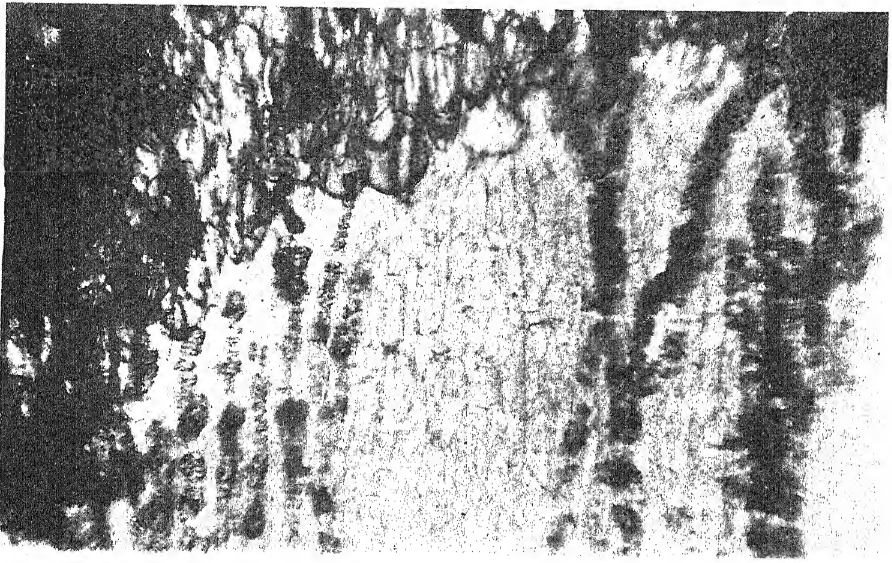




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# Observations on the Fat Metabolism of Leaves.

## II. Fats and Phosphatides of the Runner Bean (*Phaseolus multiflorus*).

BY

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AND

A. C. CHIBNALL.

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### I. INTRODUCTION.

THE object of the research which is being undertaken in this laboratory into the general fat metabolism of leaves was set forth in the first paper of this series (Chibnall and Sahai, 5). Two of the problems which were shown therein to be in need of further biochemical investigation are dealt with in the present paper.

The first of these concerns the metabolism of phosphatides in the leaf. Researches of earlier investigators, to which reference will be made later, have shown that the phosphatides of seeds contain lecithin and kephalin. Channon and Chibnall (1), however, found that in the green leaf of the cabbage both of these were absent, and that they were replaced by a new phosphatide, calcium phosphatide. If the presence of the latter is of general occurrence, as a preliminary survey suggested, then the phosphatide metabolism of the leaf must differ from that of the seed, and it became of interest to find out at what stage in the development of the plant the lecithin and kephalin gave place to calcium phosphatide.

The second problem concerns the role of the fats and phosphatides in the leaf. There is ample evidence that the glycerides of 'oil-bearing' seeds are reserve food (*élément variable* according to the classification of Mayer, Schaeffer, and Terroine, cf. Leathes and Raper (7)), and that they are utilized for growth and respiration. The amount of glycerides and phosphatides in the leaf, however, is relatively small, and it is not yet known whether they also are reserve material which can be utilized under conditions of carbohydrate deficiency, or whether they are essential

components of the cell protoplasm (*élément constant*) which would persist, even if death from inanition resulted.

Chibnall and Sahai (5) attempted to throw light on this second problem by experimenting with the mature leaves of the Brussels sprout. When the detached leaves were left in the dark for periods up to eight days with their petioles in water, it was found that no significant change in either phosphatide or glyceride content took place. No general conclusions could be drawn, however, on the fate of these substances during carbohydrate starvation, because the sugars in the laminae, contrary to what was expected, increased instead of decreased during the period of isolation, the laminae having drawn on the sugar reserve of the fleshy petioles.

\* For the present investigation it was necessary to use a plant that was more suitable than the Brussels sprout for the various experiments to be performed. The runner bean (*Phaseolus multiflorus*), in which the leaf-growth is uniform and the petioles small relative to the pinnae, was chosen because previous work by Chibnall (2) on the nitrogenous metabolism had shown that isolated leaves left with their petioles in water exhibited a large breakdown of protein in five to six days, suggesting a carbohydrate deficiency which might lead to the utilization of fats and phosphatides. Furthermore a preliminary experiment showed that the leaves contained calcium phosphatide, and as previous researches of Schulze (15) and Trier (20) had already shown that the seeds contained both lecithin and kephalin, the plant was clearly a suitable one for experiments on phosphatide metabolism.

To find out at which stage the lecithin and kephalin were replaced by calcium phosphatide it was necessary to analyse the phosphatide fractions at regular intervals throughout growth. The experiments described in this paper therefore really fall into three main groupings:

(1) A detailed study has been made of the phosphatide fractions of the cotyledons, embryo axes, young germinating plants, prophylls, and pinnate leaves.

(2) To throw some light on the metabolism of the glyceride fatty acids and phosphatides the changes in character and amount of these substances have been followed throughout the life-history of the plant.

(3) Isolated mature pinnate leaves have been kept in the dark with their petioles in water to find out if either the glyceride fatty acids or phosphatides could be utilized under conditions of carbohydrate deficiency.

## II. General Experimental Methods.

*Materials used.* The runner bean (Scarlet Champion) seeds were planted about the end of April, 1932, in unmanured ground at the Physic Gardens, Chelsea. For convenience in handling and to ensure more even germina-

tion, the seeds used to provide the very large number of germinating plants required in the early stages were planted in boxes containing similar soil, which were placed on the ground alongside the main plots. Growth was normal, and at suitable time intervals the growing points were tied with coloured wool so that the leaves growing on the main axis, from which the pinnate leaves used in the experiments were collected, were of known age. Full details of the samples collected are given in Table I.

*Morphology of the plant.* The seed consists solely of the embryo surrounded by a double seed-coat. The embryo itself consists of a short axial portion, divided into a primary root (radicle) and primary shoot (plumule), and to this axis are attached two large fleshy cotyledons or seed leaves in which the whole of the reserve food is stored. On germination the radicle first emerges, penetrates the soil and develops lateral roots. The plumule develops more slowly, the portion of the stem above the cotyledons (the epicotyl) growing strongly and assuming a characteristic curved form so that it appears as a 'knee' between the cotyledons. This now straightens out, drawing the young leaves of the developing plumule from between the cotyledons and raising them above the soil. The cotyledons, which provide the material for early growth, remain below the soil. The epicotyl attains a considerable length, and the two first leaves of the young shoot begin to expand rapidly. These two leaves (the prophylls) differ in form from the leaves of the adult plant, for they consist of simple heart-shaped laminae borne on a short stalk, whereas the normal leaf is a compound leaf of three leaflets (pinnae) attached to a common petiole.

*Preparation of the ether extract.* The ungerminated seeds were opened by hand and the cotyledons and embryo axes separated. Both were readily ground in a coffee-mill to a powder and were extracted with ether. In the young germinating plants the seed-coat containing the cotyledon was first removed. The cotyledons and developing axes were then rapidly dried in an air oven at 80°, powdered, and extracted with ether. In the case of the prophylls and the ordinary pinnate leaves a batch of 500–1,000 gm. was picked on a day when no rain had fallen during the previous twelve hours, so that the laminae were free from extraneous moisture. The stems (up to the point of insertion of the three pinnae in the case of the pinnate leaves) were then removed and the fresh weight of the laminae at once determined. Two samples of 30 gm. were then placed in an oven at 108° to obtain the total dry weight, and the remainder, after rapid drying in an air oven at 80° (1–1½ hours), were powdered in the mill and extracted with ether. All ether extractions were for 24 hours in a Soxhlet apparatus, and the analyses of the extracts were made by the methods used by Chibnall and Sahai (5).

During the summer of 1931 some preliminary experiments were made on the variations of the ether-soluble material of the prophylls and pinnate

TABLE I.  
*Details of Samples used for Analysis.*  
(Plants grown in open beds unless otherwise stated.)

Series No.	Age of plants above ground in weeks.	Details of plants, &c.	Age and nature of material used.	Fresh wt. of sample extracted (grm.).	Dry wt. of sample extracted (grm.).	Wt. of ether extract (grm.).
1	—	Ungerminated seeds	Cotyledons	1000	877	16.9
2	—	Ungerminated seeds	Detached embryo axes	40	34.8	1.95
3 a	—	From seeds grown in boxes for 8 days	(a) Detached cotyledons	580	200	4.0
b			(b) Remainder of embryos	1383	129	4.1
4 a	1	From seeds sown in boxes.	(a) Detached cotyledons	850	165	2.8
b		Plants 6-7 in. high with prophylls 1 in. long (15 days germination)	(b) Remainder of plants	1100	105	4.5
c			(c) 1 week old prophylls from a different sample of plants	855	128	8.6
5 a	3	From seeds sown in boxes.	(a) Detached cotyledons	205	23	0.5
b		Plants 10 in. high with fully developed prophylls and 2 small pinnate leaves (30 days germination)	(b) Remainder of plants	875	100	3.5
c			(c) 3 week old prophylls from another sample of plants	867	97	6.9
6 a	7-8	Plants 3 ft. high; beginning to form flower buds	(a) 7 week old prophylls	1600	226	14.8
b		Plants 6 ft. high having many mature leaves. Prophylls withering	(b) 3-4 week old pinnate leaves	708	82	6.6
7	10		10 week old prophylls	256	32	1.8
8	11	Plants nearly fully grown and beginning to flower	7 week old pinnate leaves	1200	162	12.8
9	14	Plants flowering and a few pods forming	10 week old pinnate leaves	660	94.5	7.1
10	16	Flowering nearly over, very dense foliage due to secondary growth	12 week old pinnate leaves	720	124	8.3
11	20	Flowering finished, a few mature pods	15 week old pinnate leaves	340	51	5.1
12	24	Leaves beginning to become tough and turning brown	18 week old pinnate leaves	500	93	6.8
13	26	Leaves withered and dark brown	Dead pinnate leaves (20 week old)	265	256	7.7



leaves during growth, in which the ether extract was prepared by the method of Chibnall and Sahai (5), which is based on the method of treating fresh leaves used by Channon and Chibnall (1). The differences in the composition of the ether extract obtained by the two methods will be discussed more fully in a later communication; at the present time it is only necessary to record the fact that the extract from *rapidly* air-dried leaves contained a higher proportion of both crude phosphatide and fatty acids than that obtained from the fresh leaves, and it was for this reason that our procedure was changed in 1932.

### III. *Phosphatides of the Cotyledons.*

Schulze and Steiger (17) showed that extraction of finely powdered seeds with ether did not remove all of the 'lecithin' present, and recommended an extraction with hot alcohol at 60°, the solvent being subsequently removed and the residue obtained extracted with ether. The quantitative determinations of 'lecithin' in seeds and seedlings by Schulze and his school, which are criticized later, were based on the total phosphorus content of the ether extracts prepared in this way. Schulze realized, however, that all this phosphorus might not represent 'lecithin', and in later experiments (15) obtained purer (non-quantitative) preparations of phosphatides by treating the ether extract with either acetone or methyl alcohol. That from runner bean meal contained 3.44 per cent. of phosphorus, and on acid hydrolysis yielded glycerophosphoric acid, choline, and fatty acids. A little later Trier (20) obtained from the same source a preparation containing 3.51 per cent. of phosphorus, which gave on acid hydrolysis  $\beta$ -aminoethyl alcohol (colamine) in addition to the other products mentioned above, thereby proving that the seeds contained kephalin as well as lecithin.

It will be shown in a later section that treatment of the cotyledon material with hot alcohol following an exhaustive extraction with ether undoubtedly extracts a further amount of phosphatide material. Preparations thus obtained, however, are very impure and probably contain heat decomposition products. As the object of the present analysis was to find out whether the cotyledons contained a small amount of phosphatidic acid salt as well as lecithin and kephalin, we have advisedly used only the phosphatide preparations obtained from the direct ether extract.

3 kg. of powdered cotyledons yielded 50.7 gm. of ether extract and 12.6 gm. of crude phosphatide. The ether solution of the latter was almost water-clear and did not darken on being exposed to air or light for some days. (Found: C, 61.0; H, 10.1; N, 1.1; P, 3.0; Mg, 0.2; Ca, 0; ash, 10.3 per cent. The N:P ratio was 0.8.) The fractionation of the crude material follows closely the procedure of Smith and Chibnall (18), whose

theoretical analytical figures for the constituent phosphatides have also been adopted.

	C %	H %	N %	P %
Lecithin . . . . .	65.3	10.9	1.77	3.91
Kephalin . . . . .	65.6	10.6	1.91	4.24
Phosphatidic acid . . . .	66.5	10.4	—	4.52
Lead phosphatidate (ash 33%)	51.2	7.7	—	3.48

*Lecithin-kephalin fraction.* An ethereal solution (250 c.c.) of the crude material (10 grm.) was shaken four times with excess of 5 per cent. hydrochloric acid and then well washed with water. 9.3 grm. of material remained soluble in ether, so that by difference 0.7 grm. had passed into the acid and aqueous washings. These latter contained 0.42 grm. of reducing substances estimated as glucose by the method of MacLean (9), and also all the magnesium, but only 9 per cent. of the nitrogen and 4 per cent. of the phosphorus present in the original crude phosphatide. 9.0 grm. of ether-soluble material were dissolved in a minimum volume of ether in a centrifugal bottle and four volumes of acetone were added with stirring. A plastic white mass separated, and after standing at 0° overnight was centrifugalized off. Reprecipitation from a smaller volume of ether-acetone gave 5.5 grm. and the mother-liquors on treatment gave 1.3 grm. of crude lecithin-kephalin, representing 70 per cent. of the original crude phosphatide. (Found: C, 62.1; H, 10.2; N, 1.3; P, 3.0; ash, 3.3 per cent. N:P ratio was 0.96.)

The work of Levene and Rolf (8) suggested that further purification of this material would be laborious, and as the presence of both lecithin and kephalin in the runner bean seed had been proved by Schulze and Trier as mentioned above, it did not seem necessary in the present case to do more than confirm the presence of the two nitrogenous bases choline and  $\beta$ -aminoethyl alcohol. The procedure adopted followed closely that of Smith and Chibnall (18), except that choline was isolated by direct precipitation as the gold salt, and not *via* choline mercuric chloride. 2.5 grm. of the material were accordingly hydrolysed with 100 c.c. of 5 per cent. sulphuric acid for ten hours. Only 65 per cent. of the original N passed into the aqueous acid, one half of which was in the amino form. The yield of choline chloroaurate M.P. 259° was 0.3 grm., representing 90 per cent. of the non-amino-N. (Found: Au, 44.0 per cent.  $C_5H_{14}ON \cdot AuCl_4$  requires Au, 44.5 per cent.) The yield of  $\beta$ -aminoethyl alcohol chloroaurate M.P. 187° was 0.1 grm., representing 30 per cent. of the amino-N. (Found: Au, 49.4 per cent.  $C_2H_7NO \cdot HAuCl_4$  requires 49.2 per cent.) The fatty acids had an iodine value of 110 and were not characterized further.

*Phosphatidic acid.* The acetone-ether mother-liquors from the lecithin-kephalin preparation mentioned above were evaporated to dryness, dis-

solved in ether and shaken three times in succession with saturated aqueous lead acetate. The ethereal solution was well washed with water, concentrated to a small volume in a centrifugal tube and two volumes of acetone were added. On centrifugalizing, the lead phosphatide was obtained as a light brown gummy mass. It was redissolved in a minimal volume of ether and reprecipitated by the addition of three volumes of absolute alcohol. The yield was 0.25 gm., and analysis showed that it was as pure as any preparation of lead phosphatide so far obtained in this laboratory. (Found: C, 50.8; H, 8.1; N, 0.2; P, 3.5; ash, 33.0 per cent.) Furthermore, as the crude phosphatide contained no calcium, and as the amount of magnesium present was sufficient to allow of about 6 per cent. of magnesium phosphatide, it may be legitimately inferred that the phosphatidic acid of the crude phosphatide was all present as this salt to the extent of 3-4 per cent. of the total weight. The ratio of magnesium phosphatide to lecithin-kephalin was therefore about 1:20.

#### IV. *Phosphatides of the Seed Embryo Axes (Plumule and Radicle).* (Series No. 2.)

5,000 seeds gave 40 gm. of embryo axes, and these 1.95 gm. of ether extract containing 0.4 gm. of phosphatide. There was only sufficient material, therefore, for a rough separation into lead phosphatide and lecithin-kephalin fractions. The crude material analysed as follows: C, 58.5; H, 9.7; ash, 14.7 per cent. On shaking with aqueous hydrochloric acid, 0.34 gm. in ethereal solution lost only 6 per cent. of its total weight. Treatment with lead acetate in the usual way and precipitation from a relatively large volume of ether-alcohol gave 0.1 gm. of crude lead phosphatide. (Found: C, 43.2; H, 7.4; ash, 33.2 per cent.) The value for C was low, but lack of material prevented further purification. The ether-alcohol mother-liquors were taken to dryness, the material dissolved in the minimum volume of ether and two volumes of acetone added. 0.2 gm. of crude lecithin-kephalin were precipitated. (Found: C, 63.2; H, 10.7; ash, 5.8 per cent.) These results suggest that the major part of the crude phosphatide consisted of a salt of phosphatidic acid and lecithin-kephalin in the proportion of 1:2.

#### V. *Phosphatides of the 8-day old Embryo Axes.* (Series No. 3 b.)

1383 gm. of material gave 4.05 gm. of ether extract and 0.8 gm. of crude phosphatide. (Found: C, 52.2; H, 9.2; N, 1.3; P, 4.7; Mg, 2.9; Ca, 0; ash, 18.0 per cent.) Although the nitrogen content was still high it will be seen that the magnesium content is considerably greater than in

the case of the seed cotyledon phosphatide; it is also interesting to observe that at this stage calcium is still absent. 0.5 gm. of material was treated by the method given immediately preceding. The amount of material removed by the hydrochloric acid increased to 20 per cent. of the total, and this contained 25 per cent. of the total phosphorus. The weight of lead phosphatide obtained was 0.3 gm (Found: C, 53.3; H, 8.2; N, 0.4; P, 3.2; ash, 28.1 per cent.), and of crude lecithin-kephalin 0.1 gm. (Found: C, 60.6; H, 10.4; N, 1.2; P, 2.0; ash, 10.2 per cent.). The ratio of phosphatidic acid to lecithin-kephalin had therefore risen to about 3:1.

Now 56 per cent. of the total phosphorus of the crude phosphatide passed into the phosphatidic acid fraction, 17 per cent. into the lecithin-kephalin fraction, and the remainder (26 per cent.) by difference into the aqueous acid. There is no experimental evidence to suggest that the latter phosphorus was derived from organic material, and if we assume that in the crude phosphatide it bound the maximum amount of magnesium as phosphate, there still remains sufficient magnesium to warrant the definite conclusion that all the phosphatidic acid was present as magnesium phosphatide.

#### VI. *Phosphatides of the Prophylls.*

*Prophylls one week old (Series No. 4 c).*—835 gm. of fresh prophylls gave 8.55 gm. of ether extract and 0.78 gm. of crude phosphatide (analysis given in Table II). Fractionated by the method given above 0.56 gm. gave 0.4 gm. of lead phosphatide (Found: C, 49.1; H, 7.6; N, 0.2; P, 3.2; ash, 30.5 per cent.) and only 0.07 gm. of very crude lecithin-kephalin (Found: C, 65.3; H, 10.3; N, 0.7; P, 1.3; ash, 8.8 per cent.). 23 per cent. of the phosphorus present in the crude phosphatide was washed out with acid, and 64 per cent. was present as phosphatidic acid. These two amounts combined are roughly equivalent to the total magnesium and calcium, but the distribution of the metals in the two salts can only be inferred. Since, however, the maturer prophylls and the pinnate leaves contain only calcium and no magnesium phosphatide, it seems only reasonable to assume that the major part of the calcium in these young prophylls was present also as phosphatide. The amount of calcium, however, is insufficient to account for all the phosphatidic acid, so that some magnesium phosphatide must also have been present.

*Prophylls three weeks old (Series No. 5 c).*—867 gm. of fresh leaves gave 6.9 gm. of ether extract and 1.1 gm. of crude phosphatide. At this stage in the research the procedure of Smith and Chibnall (18) for fractionating the phosphatides, which is described in more detail in a later experiment dealing with the pinnate leaves, was used. 0.6 gm. of crude phosphatide gave 0.08 gm. of lecithin-kephalin (Found: C, 53.2; H, 9.2;

N, 1.0; P, 2.8; ash, 12.7 per cent.) and 0.4 grm. of lead phosphatide (Found: C, 50.0; H, 7.7; N, 0.03; P, 3.5; ash, 32.9 per cent.).

*Mature prophylls.* Analyses of the crude phosphatides are given in Table II.

TABLE II.

*Analyses of the Crude Phosphatides of the Prophylls and Pinnate Leaves.*

	Series No. of sample.	Age of leaf in weeks.	C %	H %	N %	P %	Mg %	Ca %	Ash %
Prophylls.	4 c	1	54.4	8.6	0.4	4.4	1.4	2.9	21.9
	5 c	3	55.9	8.7	0.5	3.8	0.6	4.1	19.2
	6 a	7	56.7	8.3		3.6	0.0	5.1	15.9
	7	10	56.5	8.7					14.9
Pinnate Leaves.	6 b	3	56.5	8.9	0.3	3.7	0.0	5.5	18.4
	8	7	54.2	8.5	0.7	3.0	0.0	4.2	16.3
	9	10	55.3	8.5		4.0			18.3
	10	12	53.8	8.1		4.4		3.9	16.7
	12	18	56.1	8.3		4.4		4.2	16.4
	13	20	54.6	7.9		5.5		4.6	16.7

#### VII. *Phosphatides of the Pinnate Leaves.*

The analyses of the crude phosphatides are given in Table II. It will be seen that the composition remains fairly constant, and that magnesium is in every case absent. For further characterizations of the constituent phosphatides a large preparation of crude material from leaves seven weeks old (Series No. 8) was fractionated by the method used by Smith and Chibnall (18) for the phosphatides of cocksfoot. Full particulars are given in Table I of that paper (p. 1347), and to save repetition of practical details the fractions obtained in the present case are given the corresponding letter.

25 kg. of fresh leaves yielded on treatment 263 grm. of ether extract, and 31.0 grm. of crude phosphatide (Analysis given in Table II (8)). 30 grm. of the material were treated with hydrochloric acid, when 16 per cent. passed into the aqueous phase. This contained all the calcium, 27 per cent. of the phosphorus, and 20 per cent. of the nitrogen in the original phosphatide. In addition, reducing substances estimated as glucose by the method of MacLean (9) were present to the extent of 5 per cent. of the crude phosphatide.

*Acetic acid soluble lecithin-kephalin (Fraction F).* This material weighed 2.7 grm. and represented 9 per cent. of the original crude phosphatide. (Found: C, 63.1; H, 9.3; N, 1.1; P, 2.3; ash, 5.3 per cent. N : P ratio was 1 : 1.06.) The low percentages of nitrogen and phosphorus showed that the substance contained some nitrogen- and phosphorus-free

material. 2.5 grm. were hydrolysed with 2.5 per cent. sulphuric acid for 8 hours when 12 mg. of nitrogen, all in the amino form, passed into the aqueous acid.  $\beta$ -aminoethyl alcohol chloroaurate M.P. 189–191° with previous softening at 187° was readily isolated. The fatty residue on further saponification with alcoholic potash for 2 hours yielded 1.45 grm. of fatty acids (mol. wt. 284), representing only 58 per cent. of the original material, whereas lecithin and kephalin should yield 69 per cent. and 75 per cent. respectively. It would appear that this fraction contained kephalin and other products whose composition has not been determined.

*Acetic acid insoluble fraction (Fraction G).* This had a similar composition to that obtained from cocksfoot, and was probably a product of heat decomposition formed during the drying of the leaves. The weight was 3.5 grm. (Found: C, 51.5; H, 8.4; N, 1.2; P, 1.7; ash, 3.2 per cent.)

*Phosphatidic acid (Fraction J).* The crude phosphatidic acid (fraction E) weighed 18.3 grm., representing 61 per cent. of the original crude phosphatide. 17.5 grm. of the material on treatment with lead acetate gave 13.7 grm. of lead phosphatide and later 8.4 grm. of phosphatidic acid, representing 33 per cent. of the original crude phosphatide. (Found: C, 65.0; H, 10.3; N, 0.07; P, 4.3; ash, 3.7 per cent.) The mol. wt. by titration (dibasic acid) was 675, whereas the computed value of Smith and Chibnall requires 686. On saponification with strong alcoholic potash for two hours 0.82 grm. yielded 0.615 grm. of fatty acids (I.V. 132: mol. wt. 280), representing 91 per cent. of theory. The mother-liquors from the lead phosphatide precipitation gave 1.8 grm. of mixed glycerides (Fraction P) and 2.2 grm. of free fatty acids (Fraction N). These were similar to the products from cocksfoot, and were not further characterized.

TABLE III.

*Compositions of the Crude Phosphatides from Various Leaves (in percentages of total crude phosphatide).*

	Material soluble in HCl.	Phospha- tidic acid.	Lecithin- kephalin.	'Cuorin' (Fraction G).
Cabbage (Extract made by method of Chibnall and Channon) . . .	24	50–60	—	—
Runner bean (ditto) . . .	30	31	—	—
Cocksfoot grass (Extract made from dried leaves) . . .	45	10	9	9
Runner bean (ditto) . . .	16	30–35	9	12

It will be seen that the phosphatides of the pinnate leaves are similar to those of cocksfoot, and differ only in the relative amount of phosphatidic acid salts present, which approaches that found by Channon and Chibnall (1) in green cabbage leaves. Table III shows the composition

of these three leaf phosphatides, which are the only ones that have yet been examined. It was mentioned earlier that the amount of crude phosphatide obtained from rapidly dried bean leaves was very much greater than that obtained by the fresh method of Channon and Chibnall. For comparison, a second (and earlier) analysis of the bean (pinnate) leaf phosphatide is also given in Table III, showing that the increase in amount of crude phosphatide obtained from the dried leaves was not due to the presence of extraneous non-phosphatide material. The phosphorus removed by shaking with hydrochloric acid seems to be of inorganic origin, and until definite evidence to the contrary has been obtained it cannot be considered 'phosphatide-phosphorus'.

### VIII. *Discussion of Results of Phosphatide Analyses.*

*Composition of the phosphatides.* It will be seen from the experimental data given above that the apparent sharp distinction between the phosphatides of seeds, which were known to contain lecithin and kephalin, and of leaves, as exemplified by the calcium phosphatide found in cabbage leaves, is one of degree only. The observations of Schulze (15) and Trier (20) that the cotyledons (bean meal) contain lecithin and kephalin have been confirmed; in addition we have been able to show that a small but definite amount of magnesium phosphatide is present and that calcium phosphatide is absent. The small embryo axis contains the same three phosphatides, but the proportion of magnesium phosphatide is greater, as shown more clearly in Table IV. In the early days of germination the

TABLE IV.

*Approximate Compositions of the Crude Phosphatides obtained from the Runner Bean at Various Stages of Growth.*

(The figures given are percentages of the crude phosphatide phosphorus present in each fraction.)

Description of Sample.	Soluble in HCl.	Lecithin- kephalin.	Mg Phosphati- date.	Ca Phosphati- date.
Ungerminated seed cotyledons . . . . .	5	68	3.5	—
Ungerminated embryo axes . . . . .	5	55	32	—
8 day old seedling after removal of cotyledons . . . . .	25	9	42	—
Mature prophylls . . . . .	25	4	2	50
Mature pinnate leaves . . . . .	27	7	—	48

proportion of magnesium phosphatide greatly increases. Calcium phosphatide does not appear until the young prophylls expand, after which stage the magnesium is rapidly replaced by calcium, so that the phosphatides of the mature prophylls and both young and mature pinnæ consist chiefly of calcium phosphatide. A small but definite amount of lecithin-

kephalin, however, seems to persist throughout. In a later section we suggest that this curious difference in phosphatide balance between the cotyledons and the green parts of the plant may be due to the fact that the phosphatides of the cotyledon are essentially reserve food which is rapidly utilized on germination, whereas the phosphatides of the leaves are integral parts of the cell protoplasm. From the results given in Table IV it might be suggested that the lecithin-kephalin of the seed was the precursor of the phosphatidic acid in the growing organs. That such a conclusion is erroneous, however, can be readily shown if precautions are taken to compare, not the relative, but the actual amounts of each phosphatide at various stages of growth.

#### IX. *The Total Phosphatide present in the Various Organs.*

It is necessary here to recall the fact, mentioned earlier, that Schulze and his co-workers have shown that ether does not extract all the phosphatide from seed material. Our own results confirm this, and as we wished to trace the quantitative changes which take place during germination we have submitted the ether-extracted cotyledon residues to the hot-alcohol treatment recommended by them. The material was treated three times successively with absolute alcohol at 60° for three hours, and the solvent removed *in vacuo*. The residue thus obtained was extracted repeatedly with boiling ether, when there remained a gum which was readily soluble in water. The ethereal solution was reduced to a small volume, treated with three volumes of acetone, and the precipitate of crude phosphatide centrifugalized off. The amounts of phosphorus in the aqueous solution, crude phosphatide, and ether-acetone mother-liquors are given in Table V.

TABLE V.

*Showing the Amount of Phosphorus extracted by Warm Alcohol from the Ether-extracted Cotyledons at Various Stages of Growth.*

(The weights given are in mg. per 1000 seeds or seedlings.)

Description of sample.	Total phosphorus extracted	Phosphorus insoluble in ether and soluble in water.	Phosphorus in acetone-ether precipitate.	Phosphorus in acetone-ether mother-liquor.	Phosphorus in lecithin-kephalin fraction.
Cotyledons of un-germinated seeds	319	14	190	115	96
Cotyledons of 8 day old seedlings	189	78	102	9.4	37.4
Cotyledons of 15 day old seedlings	90.4	39	37	14.4	15.5

The crude phosphatides mentioned above were purified by the method adopted for the similar products derived from the direct ether extract.



Details are given in Table VI. Much material was removed by shaking with HCl, and the lecithin-kephalin fractions obtained were clearly impure. The acetone mother-liquor which should have contained any phosphatidic acid consisted of very impure products that were not further characterized. Reference to Table V will show that the amount of true phosphatide (lecithin-kephalin) phosphorus extracted by the hot alcohol is only about one-quarter of the total, showing clearly that the results of Schulze and Stoklasa quoted later have very little quantitative significance. In connexion with the data discussed in the next section it may be stated here that the amount of glyceride and unsaponifiable material extracted by hot alcohol from the ether-extracted cotyledon material is negligible, and that no significant amount of these substances, or of phosphatide, is extracted by the same solvent from the ether-extracted leaf-material. In order that the true value for lecithin-kephalin present in the cotyledons should appear in Table VII, the additional amount extracted by warm alcohol has been incorporated.

TABLE VI.

*Analyses of the Crude Phosphatides extracted by Warm Alcohol.*

(The weights given are in grm. per 1,000 seeds or seedlings.)

Description of sample.	Crude phosphatide before treatment with HCl.		Crude phosphatide after treatment with HCl.			'Lecithin-kephalin'.					Lecithin-kephalin obtained from direct ether extract (see text).
	wt. (grm.)	P %	wt. (grm.)	P %	N %	wt. (grm.)	C %	H %	N %	P %	
Cotyledons of ungerminated seeds . . .	8.2	2.3	5.0	3.1	0.9	2.6	57.7	9.4	1.2	3.7	5.2
Cotyledons of 8 day old seedlings . . .	6.2	1.7	2.5	3.1	—	1.1	58.0	9.7	1.4	3.4	1.0
Cotyledons of 15 day old seedlings . .	3.1	1.2	1.1	3.0	—	0.5	55.4	10.2	1.3	3.1	0.6

X. *Changes in the Phosphatides and Glyceride Fatty Acids during Germination and Growth.*

*Methods of comparison of samples.* In the case of the seed and germinating seedling the only satisfactory basis of comparison is that based on the weight of the material present in a given number of seeds or plants. A comparison based on a percentage of the dry weight is open to the objections that (1) during germination there is a loss of weight due to respiration, (2) at the onset of leaf formation the dry weight increases through photosynthesis, so that if the substance under investigation is not

concerned directly with respiration or photosynthesis it will at the former stage exhibit an artificial enrichment, and at the latter stage an artificial diminution. Further reference will be made to this in a later section dealing with the results of earlier workers.

But with the pinnate leaves the problem is less readily solved. In a discussion on the diurnal variations in leaves, in which the time interval is so short that changes due to growth can be neglected, Chibnall (3) advanced reasons for suggesting that a better comparison was obtained if the variant were expressed as either a weight in terms of a certain number of leaves or as a percentage of the leaf fresh weight than if it were expressed as a percentage of the leaf dry weight—the method, incidentally, which had been generally used by earlier workers. More recently, Mason and Maskell (10) have suggested that in the case of sugars, which show a large diurnal variation, a better method of comparison was that based on ‘residual dry weight’. In the present case we are concerned with the changes in the pinnate leaves due to growth, so that some of the arguments used in the above discussions are invalid. Growth of the individual pinnae depends on factors such as sunlight, which cannot be readily controlled, so that comparison on a basis of a given number of leaves is not to be recommended. As the cell wall material of the leaf cells thickens during growth, there is, on this account, a continuous increase in dry weight which may effectively mask a small variation in some other constituent. Somewhat arbitrarily then, for lack of a better method, we have expressed the seasonal changes of the pinnae in terms of the fresh leaf weight.

*Discussion of results.* The results of the experiments dealing with the germination of the seed and the early development of the prophylls, expressed in terms of 1,000 seeds or seedlings, are given in Table VII. It will be observed that the cotyledons contain 99 per cent. of the ether extract of the seed, and of this 90 per cent. is made of phosphatides and glyceride fatty acids. Although the embryo axis is relatively much richer in fatty material than the cotyledons, the actual amount present in 1,000 seeds is very small, and about 75 per cent. of it is made up of fatty acids and phosphatides. The iodine value of the acids is about 20 lower than that in the cotyledons.

During germination there is a small decrease in total dry weight, due presumably to respiration, and at the same time a transfer of material from the cotyledons to the growing axis. At the end of 8 days the glyceride fatty acids of the cotyledons have fallen from 18.4 to 16.5 gm. per 1,000 plants, and the iodine value from 160 to 141, while those of the axis have increased from 0.2 to 1.2 gm. per 1,000 plants with again a fall in iodine value from 141 to 120. At the same time there is a large fall in the amount of phosphatide in the cotyledons, from 8.0 to 2.1 gm. per 1,000 plants, with an increase in the growing axis from 0.1 to 0.35 gm. per 1,000 plants.

TABLE VII.  
*Analyses of the Ether Extracts from Seeds and Germinating Plants.*

(The weights given are in grm. per 1,000 seeds or seedlings.)

Description of sample.	Total dry weight.	Total ether extract.	Chlorophyll.	Total crude phosphatide.	Lecithin-kephalin.	Magnesium phosphatide.	Calcium phosphatide.	Glyceride fatty acids.	Unaponifiable material less phytol from chlorophyll.	Wax.	I. V. of glyceride fatty acids.	Neutralization value of glyceride fatty acids.	I. V. of unaponifiable material.
Ungerminated seed:													
cotyledons . . .	1200	29.1	0.0	7.7	$\begin{Bmatrix} 5.2 \\ 2.6 \end{Bmatrix}^*$	0.2	0.0	18.4	2.5	0.0	160	197	121
Ungerminated seed:													
embryo axes . . .	7.4	0.4	0.0	0.1	—	—	0.0	0.2	0.1	0.0	141	194	101
After 8 days' germination:													
cotyledons . . .	1100	22.1	0.0	1.4	$\begin{Bmatrix} 1.0 \\ 1.1 \end{Bmatrix}^*$	—	0.0	16.5	1.6	0.0	141	190	—
remainder of plants . .	88	2.7	0.0	0.5	0.1	0.25	0.0	1.2	0.6	0.0	120	202	80
After 15 days' germination:													
cotyledons . . .	600	9.9	0.0	1.0	$\begin{Bmatrix} 0.6 \\ 0.5 \end{Bmatrix}^*$	—	—	6.4	1.0	—	137	193	—
prophylls . . .	127	8.5	1.0	0.9	0.1	0.1	0.5	4.6	1.0	0.1	166	197	122
stems and roots . . .	323	10.8	0.0	2.0	—	—	—	4.0	2.5	—	—	—	—
After 30 days' germination:													
cotyledons . . .	170	3.6	0.0	0.5	0.3	—	—	2.4	0.4	—	—	—	—
prophylls . . .	525	37.4	4.6	7.2	0.6	0.0	4.3	15.0	4.8	0.5	182	198	137
pinnate leaves . . .	252	20.0	2.1	3.1	0.3	0.0	1.3	7.1	3.1	0.6	185	197	150
stems and roots . . .	1263	14.1	0.0	2.3	—	—	—	4.7	4.2	—	—	—	—
Plants 7-8 weeks old:													
prophylls . . .	670	45.0	5.0	6.0	0.5	0.0	3.6	13.5	11.5	1.8	180	198	195
pinnate leaves . . .	1160	93.0	10.0	14.0	0.7	0.0	5.6	33.0	14.0	2.6	185	197	150

\* Additional material obtained from hot-alcohol extract, see text.

It would appear that the small amount of magnesium phosphatide in the cotyledons has been translocated to the growing parts, but the major part of the lecithin-kephalin has been metabolized. Now the glyceride fatty acids which have disappeared from the cotyledons must have been highly unsaturated (I.V. > 160) (cf. Ivanov, (6)), whereas those which have appeared in the growing parts are less unsaturated (I.V. < 120). It is possible that translocation of the former, followed by saturation, has occurred, but it seems more likely that if translocation of fatty material as such can take place in the plant the latter acids have come from the lecithin-kephalin of the cotyledons having acids of I.V. < 110, thus suggesting that these phosphatides have first been translocated to the growing organs and have then been metabolized—in part to give fatty acids and hence glycerides, and in part to provide other products of non-fatty nature.

At the end of 15 days' germination the total dry weight of 1,000 plants has again decreased. That of the cotyledons has fallen to half the original value, and while there is only a small decrease in the amount of phosphatide, there is a decrease of 10 grm. per 1,000 plants in fatty acids. The young prophylls have expanded, and in them for the first time calcium phosphatide appears. They contain also a small amount of magnesium phosphatide, which disappears on further growth. Although no significance can at present be attached to the observation, it is interesting to note that the disappearance of (fat-soluble) magnesium coincides with the production of chlorophyll. Reference to Table VII will show that the loss of fatty acids from the cotyledons is balanced by a nearly corresponding gain in the growing organs. There is little evidence, however, to show whether the transfer of material took place in the form of glycerides or fatty acids, followed by desaturation in the prophylls, or whether the cotyledon acids were degraded to simpler (water-soluble) products, which, after translocation to the growing points, have been used for the synthesis of new unsaturated acids. If degradation of the fatty acids by the usual process of oxidation has indeed occurred, then the resulting products must have been water-soluble, for the neutralization value of the remaining fatty acids definitely excludes lower (fat-soluble) homologues such as myristic, lauric, and decoic acids. It is also worthy of note that synthesis of highly unsaturated acids from simpler (photosynthetic) products undoubtedly takes place in the maturer prophylls, so that it might reasonably be argued that some such synthesis has also taken place in the present instance, at least in the young prophylls.

It will be readily seen that a satisfactory interpretation of the above data concerning the movement of fatty materials on germination cannot be put forward until more definite evidence is available as to whether glycerides, higher fatty acids, and phosphatides can be translocated in the vessels

or whether they can diffuse from cell to cell—so further discussion at the present time seems premature.

In the 30 day old seedlings the prophylls are fully grown, and the young pinnae are developing. The effect of photosynthetic activity is shown by the twofold increase of total dry weight. The cotyledons have shrunk and now make up only one-tenth of the total weight, but there has been only a relatively small loss of fatty acids and phosphatides. Rapid synthesis of calcium phosphatide and of highly unsaturated glyceride fatty acids has taken place in both the prophylls and pinnae, as shown in Table VII.

The observation that the iodine value of seed fatty acids falls on germination confirms the earlier work of Ivanov (6), although in his experiments no attempt was made to distinguish between the acids derived from the glycerides and those derived from the phosphatides. To compare the changes which we have found in the phosphatide content of seeds and seedlings during germination with those of earlier workers we have, as the necessary data were available, recalculated some results to conform with experimental procedure adopted by them. Table VIII shows the amount of phosphatide in the seeds and seedlings calculated by three different methods:

(1) From the weight of true phosphatide isolated in the present research, and expressed as a weight per 100 seeds or seedlings.

(2) From the phosphorus content of the ether extract as prepared by the method of Schulze and Steiger (17), and expressed as a percentage of the dry weight.

(3) From the phosphorus content of the ether and three hot-alcohol extracts made in the present research, and expressed as a weight per 100 seeds or seedlings.

Method 2 gives values that are higher, but of the same order as method 1, so that the fall in phosphatide content on germination found in the present research confirms in a general way the results of Schulze (16) with etiolated *Vicia sativa*, of Schulze and Steiger (17) with etiolated *Lupinus luteus*, and of Prianischnikoff (13) with *Vicia sativa* grown in light, all of whom have used method 2. Method 3 gives fictitiously high values for the seedlings, due to the extraction of water-soluble phosphorus by the boiling alcohol. Except that he employed eight instead of three successive extractions with boiling alcohol, this is the method used by Stoklasa (19), and his anomalous results, showing an increase of phosphatide on germination, are undoubtedly due to the large amount of non-phosphatide phosphorus extracted by the alcohol from the seedlings.

TABLE VIII.

*Showing the Amount of Phosphatide in Seeds and Seedlings calculated by different Methods. (See Text.)*

Method No.	Ungerminated seeds.	8 day old seedlings.	15 day old seedlings.
1.	10.1	2.9	5.4
2.	14.1	4.1	7.6
3.	15.9	7.7	9.1

#### XI. *The Role of Fatty Acids and Phosphatides in the Mature Leaves.*

As the plant develops, the fully grown prophylls slowly lose part of their fatty acids and phosphatides, and when incipient chlorophyll degeneration appears at the end of about ten weeks the loss in each case has amounted to about one-third. The fall in the iodine value of the fatty acids suggests that the more unsaturated acids have been preferentially translocated or metabolized. The amount of unsaponifiable material, from which the phytol derived from the chlorophyll has been deducted, shows a continuous increase, as does also the wax, suggesting that both of these fractions are by-products of metabolism which slowly accumulate (cf. Sahai and Chibnall (14)).

Development of the pinnae takes place with remarkably little change in the relative amounts of fatty acids and phosphatides present, as shown in Table IX. The unsaponifiable material and wax again increase with age of the leaf, leading to the same conclusion as that stated above.

To obtain some evidence as to whether the leaf fatty acids and phosphatides, although present in relatively small amounts, can function as reserve food under conditions of carbohydrate starvation, batches of leaves have been kept in the dark for some days with their petioles in water. Details concerning the collection and treatment of the control and starvation samples were similar to those of the earlier experiments of Chibnall (2) on the nitrogenous metabolism of the runner bean leaf. For analysis, the laminae were detached from the petioles and rapidly dried in an air oven at 80°. The analytical methods were those of Chibnall and Sahai (5), except that protein was calculated ( $N \times 6.25$ ) from the value for total nitrogen insoluble in boiling water.

The first experiment was carried out with young but fully grown pinnate leaves picked in July. The control sample immediately after picking weighed 1,600 gm. and that for the starvation experiment 1610 gm., made up in each case of 1,280 gm. of laminae, with 320 gm. and 330 gm. of petiole respectively. The starvation sample was kept in the dark at about 17°, with the petioles in water. At the end of eight days the laminae were removed and analysed; they were completely flaccid and chlorophyll degeneration appeared to be complete.

TABLE IX.

*Analyses of the Ether Extracts from the Prophylls and Pinnate Leaves.*

(gm./1000 gm. fresh leaves.)

Series No. of sample.	Age of leaves in weeks.	Total dry weight of leaves.	Total ether extract.	Chlorophyll.	Carotene.	Xanthophyll.	Total crude phosphate.	Wax.	Glyceride fatty acids.	Unsaponifiable material less phytol from chlorophyll.	I. V. of glyceride fatty acids.	Neutralization value of glyceride fatty acids	I. V. of unsaponifiable material.
4c	1	153	10.2	1.2	0.07	0.14	1.1	0.08	5.6	1.2	166	199	122
5c	3	112	8.0	1.0	0.07	0.14	1.5	0.10	3.2	1.1	182	198	137
6a	7	134	9.0	1.0	0.07	0.10	1.2	0.36	2.7	2.3	180	198	195
7	10	126	7.1	0.7	0.05	0.06	0.9	0.21	2.0	2.8	143	192	211
6b	3	116	9.3	1.0	0.08	0.12	1.4	0.26	3.3	1.5	185	197	150
8	7	135	10.7	2.4	0.08	0.18	1.3	0.42	4.0	1.0	189	198	152
9	10	143	10.9	2.3	0.10	0.18	1.5	0.43	3.5	1.8	190	192	176
10	12	171	11.4	—	—	—	1.4	0.43	3.5	3.2	166	192	180
13	20*	967	29.4	1.5	0.18	0.44	1.0	3.6	6.1	9.2	92	194	101

\* Dead Leaves.

In the second experiment maturer leaves were picked in August. Both the control and starvation samples weighed 900 grm. made up of 675 grm. of laminae and 225 grm. of petioles. The period of starvation was five days, and when the laminae were detached for analysis they were turgid and showed no signs of chlorophyll degeneration. In the third experiment, old but healthy leaves were picked in October. The initial weight of both control and starvation samples was 440 grm., made up of 340 grm. of laminae and 100 grm. of petioles. The period of starvation was five days, and when collected for analysis the laminae were becoming flaccid, but showed no sign of chlorophyll degeneration.

TABLE X.

*Showing the Change in the Distribution of Nitrogen and Sugars in Leaves placed with their Petioles in Water.*

(Weights given are grm. per 1,000 grm. of original dry leaf material.)

	Control leaves picked in July.	Leaves kept 8 days in the dark.	Control. leaves picked in August.	Leaves kept 5 days in the dark.	Control leaves picked in October.	Leaves kept 5 days in the dark.
Reducing sugars . .	24.5	13.9	26.5	18.7	23.6	20.3
Non-reducing sugars	41.5	17.1	29.2	4.4	40.8	7.6
Total sugars . . .	66.0	31.0	55.7	23.1	64.4	27.9
Total nitrogen . . .	46.7	49.4	41.2	44.6	44.1	45.1
Water-soluble nitro- gen . . . . .	11.2	34.0	11.2	18.8	10.3	18.7
Protein nitrogen . .	35.5	15.4	30.0	25.8	33.8	26.4

The changes in the various constituents of the leaves kept in the dark are shown in Tables X, XI, and XII. It will be observed that at the end of five days the sugar concentration has fallen to less than half the control value, and that no further fall occurs when the period is extended to eight days, suggesting that a minimum sugar tolerance had been achieved in the shorter period (cf. Parkin (12)), and that during the extra three days the leaves were suffering from carbohydrate deficiency. It is true that the eight-day period showed a higher concentration of non-reducing sugars than the five-day, but this difference might well be attributable to variation in sugar metabolism with leaf age, for the former leaves were much younger than the latter.

At the end of five days there is a fall in the protein nitrogen with a corresponding increase in simpler water-soluble products. The older (October) leaves exhibit a greater protein breakdown than the younger (August) leaves, which is in keeping with the observations of Mothes (11). Increasing the period of isolation to eight days leads to a further very rapid breakdown of protein, which is reduced in amount to considerably less than half the control value.



TABLE XI.

*Showing the Changes in the Composition of the Ether Extract in Leaves placed with their Petioles in Water.*

(Weights given are in grm. per 1,000 grm. of original dry leaf material.)

	Control leaves picked in July.	Leaves kept 8 days in the dark.	Control leaves picked in August.	Leaves kept 5 days in the dark.	Control leaves picked in October.	Leaves kept 5 days in the dark.
Total ether extract . .	79.0	35.3	74.3	62.6	100.2	77.2
Chlorophyll . . . .	17.4	2.7	8.7	2.9	6.4	3.6
Carotene . . . . .	0.6	0.5	0.6	0.3	0.8	0.6
Xanthophyll . . . .	1.3	0.8	0.7	0.3	0.9	0.8
Crude phosphatide . .	9.5	3.6	11.3	9.5	15.2	11.9
Fatty acids . . . . .	29.4	8.1	23.7	17.1	30.4	22.6
Wax . . . . .	3.1	2.9	3.6	2.7	4.0	4.1
Unsaponifiable ma- terial . . . . .	13.3	13.3	15.4	15.2	24.0	21.7
Sterols . . . . .			2.0	2.3	2.8	3.2
I. V. of fatty acids .	189	129	118	138	148	133
Neutralization value of fatty acids . . .	198	192	189	189	202	187
I. V. of unsaponifi- able material . . .	152	148	156	149	167	166

The phosphatides show a small fall at the end of five days, and a very much greater fall at the end of eight days. From the data given in Table XII it is clear that the breakdown of phosphatide runs parallel to that of protein, for the protein-phosphatide ratio remains constant in each of the starvation experiments. The fatty acids, on the contrary, show no such close correlation with the proteins. In the younger (August) leaves there is already a loss of one quarter in the first five days of starvation, and this increases to three-quarters at the end of eight days. The results suggest that the fatty acids are utilized much more rapidly and completely than either the proteins or phosphatides.

Summarizing the above results, it would appear that when detached mature leaves are placed in darkness with their petioles in water all the available carbohydrate is metabolized in under five days. Fatty acids are utilized somewhat more slowly, and both proteins and phosphatides only to a small extent. If the leaves are kept for a further period in darkness the deficiency of available carbohydrate leads to a rapid mobilization of fatty acids, phosphatides and proteins, so that at the end of eight days in all, when the leaf cells are dying, the fatty acid content has been reduced in amount to about one quarter, and both proteins and phosphatides to nearly one-third of their original values.

Although they have not been utilized as readily as the sugars, it

would appear that the fatty acids of the glycerides have, in part at least, functioned as reserve material, so that they might, if necessary, be classified as *élément variable*. The same is true also of the phosphatides. Now according to Mayer, Schaeffer, Terroine, and their colleagues an *élément*

TABLE XII.

*Showing certain Relationships between Freshly Picked Leaves and those kept with their Petioles in Water for Varying Periods.*

(Weights given are in grm. per 1,000 grm. of original dry leaf material.)

	Control leaves picked in July.	Leaves kept 8 days in the dark.	Control leaves picked in August.	Leaves kept 5 days in the dark.	Control leaves picked in October.	Leaves kept 5 days in the dark.
Total protein . . . .	222	96.5	187.5	161	211	165
Crude phosphatide . .	9.5	3.6	11.3	9.5	15.2	11.9
Fatty acids . . . .	29.4	8.1	23.7	17.1	30.4	22.6
Ratio protein/phosphatide . . . .	23.4	26.8	16.6	17.0	13.9	13.8
Ratio protein/fatty acids . . . . .	7.5	11.9	7.9	9.4	7.0	7.3

*constant* is an essential component of the cell protoplasm which is not diminished in amount, even when death from inanition results. Proteins and phosphatides are generally regarded as the chief components of protoplasm. Previous researches on the leaf proteins (Chibnall and Grover (4)) have shown that the major part is present in the cytoplasm, and the present research has shown that, on starvation, breakdown of protein goes hand in hand with breakdown of phosphatide. The conclusion seems justified that both the proteins and phosphatides are components of the protoplasm, and that under conditions of carbohydrate deficiency, engendered by keeping detached leaves in the dark, the rapid breakdown of protoplasm has ultimately led to the death of the cell. We, therefore, fail to find any sharp distinction between the *élément constant* and the *élément variable*. In this respect the leaf is somewhat similar to muscular tissue in the animal, in that a residual amount of the total 'fat' persists after death from starvation.

Table XIII shows that the phosphatides of the seed can be considered to be in large part reserve food which is rapidly metabolized on germination, and it is perhaps this difference in the part that the phosphatides of the cotyledons and green leaves play in the life-processes of the plant which accounts for the difference in the lecithin-kephalin to phosphatidic acid balance, which has been so clearly demonstrated in the present research.

TABLE XIII.

*Protein-phosphatide Ratios in the Cotyledons of the Seeds and Germinating Seedlings.*

(Weights given are in grm. per 1,000 seeds or seedlings.)

	Cotyledons of seed.	Cotyledons of 8 day old seedlings.	Cotyledons of 15 day old seedlings.
Weight of protein . . .	234	179	76
Weight of phosphatide after shaking with HCl . . .	9.9	2.4	1.5
Ratio protein/phosphatide .	23.6	75	50

It will be seen from Table XI that the wax and unsaponifiable material have remained unchanged during starvation, confirming the view previously expressed that they are end-products of metabolism. The sterols also show no significant change, and are, therefore, not utilized on starvation. Sufficient data has not been accumulated throughout the research to throw light on any possible relationship between the sterols and the fatty acids.

## XII. SUMMARY.

1. To throw further light on the metabolism of phosphatides in the plant an extensive investigation has been made into the chemical nature of the phosphatides present in the seed cotyledons, embryo axes, young germinating plants, prophylls, and pinnate leaves of the runner bean.

2. All the organs contain lecithin and kephalin, and in progressively smaller amounts from the cotyledons to the pinnate leaves.

3. The cotyledons and embryo axes also contain a small amount of magnesium phosphatide. On germination the relative amount of this salt in the growing organs increases rapidly until the prophylls start to expand, when the magnesium is slowly replaced by calcium. Calcium phosphatide is the chief phosphatide of the mature prophylls and pinnate leaves.

4. It is interesting to observe that the replacement of (fat-soluble) magnesium by calcium takes place at the onset of chlorophyll synthesis.

5. On germination the disappearance of phosphatides from the cotyledons is much more rapid than the appearance of phosphatides in the growing organs. Consequently the seed phosphatides must function chiefly as reserve food.

6. Mature pinnate leaves have been kept in the dark with their petioles in water for periods longer than that required to consume all available reserve carbohydrate. The glyceride fatty acids are metabolized fairly rapidly, suggesting that they can function on reserve food. The phosphatides, on the contrary, disappear more slowly, and hand in hand with the protein, suggesting that they are integral parts of the protoplasm.

7. It is suggested that the difference in the part which the phosphatides of the seeds and pinnate leaves play in the life-processes of the plant may account for the difference observed in the lecithin-kephalin to phosphatidic acid balance.

8. Additional evidence is obtained to support the view already expressed that the waxes and unsaponifiable material of plants are end-products of metabolism which slowly accumulate.

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# On the Xylem Elements of Certain Fossil Pteridophyta.

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With Plate VII

THE introduction of a new method of making sections of fossil plants by Walton (12) in 1929 opened out a fresh avenue of research in minute anatomy. The old technique gave sections of such thickness that details of wall structure in these plants were seldom revealed. Kidston and Gwynne-Vaughan (8), in their work on the fossil Osmundaceae, record the disappearance of the primary tracheal wall at certain points, so that the pit cavities are vertically continuous in the middle of the wall, for some members at any rate of this family; Holden (6) describes the pit-closing membrane in the xylem elements of *Ankyropteris corrugata* Williamson sp.; and various investigators have observed fine vertical threads connecting the adjacent horizontal scalariform bars of thickening in the tracheae of some Lepidodendroid plants, but apart from these exceptions little is known of the detailed wall structure of the xylem elements of the fossil ferns and their allies.

The present work is limited to detailed observations on the wall structure of the xylem elements of some fossil Pteridophyta. A correlated study of some of the living forms is now in progress, and the question of the presence or absence of the pit-closing membrane in these plants, raised by Gwynne-Vaughan (4), Halft (5), Bancroft (1), and Wright (17), will be dealt with in a later paper.

With regard to the thin vertical threads that extend between the scalariform thickening bars of the xylem elements of some Lepidodendroid plants, there is at present a duality of conception as to their nature. Williamson (13), in 1869, describing a Lepidodendroid plant from the Coal Measures, comments upon the presence of a number of delicate, vertical lines of lignin, usually simple, less frequently branched, connecting together the contiguous transverse bars of the vessels both in young and old stems.

This author subsequently draws attention to the same feature in an Arran *Lepidodendron* (14), in *Bothrodendron mundum* Williamson sp. (15), and in young branches of *Lepidophloios Harcourtii* Witham (16).

Similar thickening threads were observed by Hovelacque (7) in 1892, in both the primary and secondary xylem of *Lepidodendron selaginoides* Sternberg.

In the same year, however, Solms-Laubach (11), drawing attention to this structure in a Culm species of *Lepidodendron*, advanced the view that the threads probably correspond with the 'Grenzhauchten' of the tracheid wall, the mid-lamella having disappeared.

Then Seward and Hill (9), in 1900, describing a Lepidodendroid stem from Dalmeny, stated that in the tracheids of both the primary and the secondary wood the position of the pit-closing membrane was occupied by numerous delicate threads extending from one thickening band to the next, and that in tangential longitudinal section the pit-closing membrane appeared to consist of a double series of irregular fine threads. It was suggested that these threads had probably been formed by the contraction and tearing of the thin membrane which originally connected the scalariform bands. Seward (10), at a later date, in discussing similar threads in the primary xylem of *L. vasculare* Binney, considered that they probably constituted the remains of the original wall, and that this might represent a stage where the intervening membrane was in process of absorption, though the possibility of the threads being the result of contraction and splitting of the membrane was not ruled out.

Finally, Zalessky (18), in 1909, describing similar threads between the scalariform thickenings in both the primary and secondary stem xylem of a species of *Sigillaria*, returned to the view held by Williamson and Hovelacque, that the threads were threads of thickening, and did not represent any part of the primary wall.

We thus arrive at two different conceptions of the nature of these fine threads. Either they are threads of thickening, developed after the formation of the primary wall, as was believed by Williamson, Hovelacque, and Zalessky, or they represent the result of some alteration of the whole or of a part of the primary wall, which was the position taken up by Seward and Hill and Solms-Laubach.

It is the object of the present work to clear up this point, and, in addition, to reveal some new features of xylem wall structure in three members of the Zygopterideae.

Sections were obtained by a development of the method described by Walton (12). A suitable acid was used to dissolve away a thin layer of the matrix in which the plant tissues were contained. The material removed was replaced with a cellulose solution, which, drying to a thin film, was peeled off and carried with it a section of the plant tissues.

In order to obtain thin, clear, film sections which could be mounted in Canada balsam, experiments were made with various solvents, cellulose compounds, and 'plasticisers'. These experiments are more fully described elsewhere (2). In the present work the solutions most generally used consisted of amyl acetate with 5 per cent. of castor oil, or of two parts of acetone to one part of amyl acetate in which 2 per cent. of triacetin was incorporated. In each case pyroxylin (gun-cotton) was used as a source of cellulose.

An excellently preserved specimen of *Stigmaria ficoides* Brongniart from the Lower Yorkian of the Yorkshire coalfield provided an introduction to the study of the nature of the delicate vertical threads so often recorded as being present in the xylem of Lepidodendroid plants. Longitudinal sections were obtained showing the wide pits in surface view with many fine threads extending vertically between the transverse thickening bands, with cross-connexions in places between the vertical threads (Pl. VII, Fig. 1). In tangential vertical sections of the walls these threads appear as two fine lines extending across the pits between the thickened scalariform rungs (Pl. VII, Fig. 2). Such sections also show that the end walls of the elements were very oblique and pitted, with the double row of fine threads between the pits here also. The threads are attached to the secondary bars very close to the points where the latter are held together by the persistent portions of the primary wall. This feature would seem to preclude any possibility of the threads having originated from the primary wall or the middle lamella.

Corroborative evidence is furnished by the transverse sections. Pl. VII, Fig. 3, illustrates a section through the middle of the bars. The thin primary wall can be seen for the whole extension of the bar with the well-developed secondary walls on either side of it. In the section shown in Pl. VII, Fig. 4, however, the double row of fine threads in the pit between the bars has been sectioned, and appears as a double row of fine dots with neither primary wall nor pit-membrane between. To the left of this figure can be seen the whole bar in section, the primary wall with the thick secondary walls on each side of it. At the point where the primary wall ends, the two rows of cross-cut threads can be seen clearly at a high focus to be on the secondary wall, and quite apart from the primary wall. Then, passing to the right of this point one can see the double row of dots representing the threads in transverse section, and there is clearly neither primary wall nor pit-membrane between them. Towards the extreme right of this figure it is seen that the wall has been torn somewhat obliquely on pulling off the film, and the threads may be traced from the dots in each of the two rows into the torn portions of the thicker secondary wall.

So far we have been dealing with the very thin, closely placed threads which are usual in these Lepidodendroid plants. Zalesky (18), in an

account of the structure of the xylem walls of a species of *Sigillaria*, stated that in some cases the threads were so thick, and at such great intervals that they could be seen distinctly by an enlargement of 75, and this author illustrates such threads in his Pl. VIII, Fig. 9, at a magnification of only 120.

A very fortunate section of a Stigmarian rootlet from a Lower Yorkian coal-ball gave thick, widely spaced threads similar to those described by Zalessky. A particularly well-preserved piece of such a wall is shown in Pl. VII, Fig. 5. The darker primary walls show distinctly in both the scalariform bars and the side walls, whilst the thick vertical threads are clearly connected to and are of the same nature as the secondarily thickened bars.

Threads of usual thinness were observed in both the primary and the secondary xylem of *L. vasculare* Binney. A transverse section of one of the walls of a primary xylem element in this form is shown in Pl. VII, Fig. 6. In the middle part of the wall, where the section has missed the secondary bars, the double row of fine dots, representing the vertical threads cut across, can be seen distinctly. The darker piece of underlying wall, which is visible in the picture, is at a deeper focus.

Pl. VII, Figs. 7 and 8, show early stages in the formation of these threads in the secondary xylem elements of *L. vasculare*. The wall illustrated in Pl. VII, Fig. 7, is of a cell close to the cambium. On one side of the thin primary wall are several small dots. Most cells in the vicinity of this one show two rows, one row on each side of the primary wall (Pl. VII, Fig. 8). In some cases the dots are quite separate from the primary wall. These dots represent the fine threads cut across, and are definitely secondary structures developed subsequently to the formation of the primary wall.

Petiole material of *Metaclepsydropsis duplex* Williamson, obtained from Professor W. T. Gordon, was next examined. This is silicified material from the Calciferous Sandstone Series, Pettycur, Fife. Professor Gordon, in his account of the structure and affinities of this form (3), states that he has never seen such perfect preservation as that shown by these silicified specimens.

Thin transverse sections of the petiole showed the pitting of the xylem walls very distinctly. The number of pits varied from one on the narrowest walls to as many as seven on the widest. Pl. VII, Fig. 9, illustrates the wall structure of one of the larger elements in transverse section. Six pits have been cut across. The boundaries of the pits are plainly seen together with the pit-closing membrane stretching across the cavity of each pit from the persistent portions of the primary wall which mark the separating boundaries of one pit from another. Furthermore, the membrane is very clearly visible in longitudinal section through the xylem walls of the



petiole in all places where the preservation is good (Pl. VII, Fig. 10). There is no distinction in this respect between the vertical side walls and the tapering end walls of the elements. Wherever the preservation is good, then the closing membrane can be seen between the pits, so that the xylem elements in this form are clearly tracheidal in nature.

The thickenings of the walls of the tracheids are reticulate (Pl. VII, Fig. 11), except in the case of the smaller tracheids, which are scalariformly thickened; the pit apertures vary in size from  $1.5\mu$  to  $6\mu$  in the narrow axis of the pit, and from  $12\mu$  to  $16\mu$  in the long axis. Pl. VII, Fig. 12, representing the wall of a large tracheid of the outer xylem, illustrates the transition between scalariform and reticulate thickening in the same element. This feature was observed by Gordon (3), dealing with the histology of the stem of this form.

The petiolar xylem of *Diptolabis Römeri* Solms-Laubach was investigated. This material was from the same source as that of *Metaclepsydropsis duplex*, but in this case was calcified, and the preservation was not quite so good as that of *M. duplex*. The pit-closing membrane could, however, be seen in both the transverse and the longitudinal sections. Pl. VII, Fig. 13, illustrates a transverse section of a tracheid wall in this form. Three sectioned pits are shown, and, as in the case of *M. duplex*, the boundaries of the pits may be seen, together with the pit-membrane stretching across the pit cavity. In this form also the xylem elements are tracheidal in nature.

Although true perforations were lacking in *M. duplex* and *D. Römeri*, they are undoubtedly present in the xylem of the petiole of *Stauropteris burntislandica* P. Bertrand. The material examined was from Pettycur, and in this case preserved in silica. The state of preservation was even better than in *M. duplex*. Pl. VII, Fig. 14, shows a perfectly clear space in each of the pits when the walls are cut vertically. The preservation is so perfect that the thickened parts of the primary wall that join together the opposite transverse bars of secondary thickening, and the secondarily thickened bars themselves, are both intact and as clear in form as in any living material of similar nature, but, in spite of the excellence of the preservation, there is no pit-membrane to be seen in any part of the sections. This is true of the end walls as well as the vertical side walls of the elements.

When the metaxylem walls are examined in face view the pitting is seen to be of the bordered scalariform type, and two or three rows of pits are present on each face of the larger elements. The darker coloured primary wall, which is markedly thick, shows distinctly through the lighter coloured secondary bars of thickening (Pl. VII, Fig. 15), and outlines the inner limits of the pit cavities. The opening of each pit into the lumen of the vessel, as outlined by the boundary of the secondary wall, is very

narrow. This feature is also indicated by the close approximation of the edges of the secondary bars, shown in Pl. VII, Fig. 14, where the walls have been cut through vertically. The narrow direction of the slit (Pl. VII, Fig. 15), which corresponds with the narrow opening of the pit into the lumen of the vessel, well shown also in a different aspect in Pl. VII, Fig. 14, measures only from  $0.5\ \mu$  in the case of the narrower slits to  $1.0\ \mu$  in the larger ones.

Pl. VII, Figs. 16 and 17, show different features of the xylem walls in transverse section. The former illustrates a cross-section of a wall passing exactly through the middle of a bar, depicting clearly the dark primary wall with the lighter coloured secondarily thickened walls on each side of it. On the other hand, Pl. VII, Fig. 17, gives the wall structure when the plane of section passes not through the centre of the bars but through the edges of the secondary bars, and showing, not primary wall, but pits between each pair of secondary bars, and there is no pit-membrane in any of the pits. The pits therefore, in this case, are true perforations, the xylem elements being true vessels in open communication with each other.

With a view to the investigation of the nature of the xylem elements in the fossil Osmundaceae, sections were made of *Osmundites Dunlopi* Kidston and Gwynne-Vaughan, *O. Dowkeri* Carruthers, *Thamnopteris Schlechtendalii* Eichwald sp., and other Osmundaceous material from New Zealand and South Africa, but, unfortunately, the perfectly preserved material of these forms which was examined by Kidston and Gwynne-Vaughan (8) was not available, and in all the material obtained by the author the state of preservation of the xylem walls was not sufficiently good to enable any reliable conclusions to be formed as to the presence or absence of the pit-closing membrane.

#### CONCLUSION.

With regard to the nature of the vertical threads in the Lepidodendroid material examined, the evidence gained from very thin transverse and vertical sections of well-preserved material indicates that these threads in the pits are threads of thickening, and form no part of the primary wall or of the middle lamella itself. The developmental stages of the secondary xylem elements obtained in *L. vasculare* (Pl. VII, Figs. 7 and 8) show clearly that these threads are formed in a double row, one on each side of the primary wall. A study of the sections of mature xylem of these forms indicates that the primary wall and middle lamella have disappeared from between the double series of threads in the pit regions, giving the appearance shown in Pl. VII, Fig. 2, where the thickened scalariform bars have been sectioned, and two distinct threads, connected to the bars, occupy

each pit. More than two such threads are seen in each pit in walls which have been sectioned obliquely, but never more than two if the bars have been squarely cut, and the sections are thin, and never, in such squarely cut sections, is there any trace of the primary wall or of the pit-membrane between the paired threads. Transverse sections of mature xylem of these forms supply confirmatory evidence that these threads do not belong to the primary wall. Similar evidence to that given by Pl. VII, Figs. 4 and 6, was obtained from all parts of the thin transverse sections of these forms.

Although there is no pit-membrane derived from part of the primary wall in these plants, yet the pit areas are occupied by a double mesh of fine, closely placed threads. In the case of the thicker, more widely spaced threads seen in the rootlet of *S. ficoides* (Pl. VII, Fig. 5), which seem to be of rarer occurrence, at least they have been seen less frequently, the mesh of double threads is much coarser.

The examination of petiolar xylem of *M. duplex* and *D. Römeri* resulted in conclusive evidence of the presence of the pit-membrane. The structures shown in Pl. VII, Figs. 9, 10, and 13, represent not isolated but prevalent examples of features that could always be observed in very thin sections of well-preserved material.

On such a fine anatomical point as the presence or absence of the pit-closing membrane in fossil plants, the state of preservation of the material is a factor of vital importance, and, in order to be of value for demonstrating the *absence* of the membrane, it is clear that any material used must be in a perfect state of preservation. With regard to the material here described, the preservation of *M. duplex* was very good, that of *D. Römeri* was not so good, but in both cases the membrane was definitely demonstrated. On the other hand, in the infinitely better preserved *Stauropteris burntislandica* there was never, in any part of the sections, the slightest evidence of the presence of the membrane. The pits were open in all cases. The perfect state of preservation increases the value of the observations on this form, and it would appear justifiable to claim that for *S. burntislandica* the pits are true perforations, and the xylem elements are therefore vessels.

Finally, I wish to thank Professor W. T. Gordon for the loan of material containing *M. duplex*, *D. Römeri*, and *S. burntislandica*, and to Mr. W. N. Edwards for the loan of the Osmundaceous material. To Professor Dame Helen Gwynne-Vaughan I am indebted for much interest and assistance in the work, and to Professor W. H. Lang for exceedingly valuable criticism and advice, and also to him and Mr. Eric Ashby for help in the photographic illustration of the paper. The work was carried out in the Department of Botany, Birkbeck College, and has been aided by the use of a microscope obtained by means of a grant from the Dixon Fund of the University of London.

## SUMMARY.

The xylem elements of certain fossil Pteridophyta have been examined.

In *S. ficoides* Brongniart and in *L. vasculare* Binney the pit areas are occupied by a double mesh of fine, closely placed, vertical threads. A Stigmarian rootlet showed thick, more widely spaced threads in the pitted areas. It is shown in all material examined that these threads are threads of thickening, and form no part of the primary wall.

The tracheidal nature of the xylem was demonstrated in petiolar material of *M. duplex* Williamson and *D. Römeri* Solms-Laubach, the closing membrane being present on both the side and the end walls of the elements.

In petiolar xylem of *S. burntislandica* P. Bertrand the pit-closing membrane is absent from the pits on all the walls, the xylem elements are therefore in open communication with each other and are vessels.

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# EXPLANATION OF PLATE

Illustrating Dr. H. Duerden's Paper 'On the Xylem Elements of Certain Fossil Pteridophyta'.

All figures from untouched photographs.

Fig. 1. Longitudinal section of the secondary xylem of *Stigmaria ficoides* showing pitting in face view and the fine vertical threads in the pits between the scalariform bars.  $\times 685$ .

Fig. 2. Tangential longitudinal section of xylem wall of *S. ficoides* showing the double row of threads in the pits.  $\times 1050$ .

Fig. 3. Transverse section passing through the middle of a single wall in the secondary xylem of *S. ficoides*. The primary and secondary walls are clearly visible.  $\times 685$ .

Fig. 4. Transverse section of secondary xylem wall of *S. ficoides*. On the left of the figure the whole thickness of the wall has been sectioned, then passing from left to right the double row of cross-cut threads are seen, and towards the extreme right the threads can be followed from each of the rows into the torn portions of the thicker secondary walls.  $\times 685$ .

Fig. 5. Vertical section of a rootlet of *S. ficoides*. The walls of the xylem elements are seen in face view. Here are seen the thicker, more widely spaced threads; these are clearly attached to the secondarily thickened bars.  $\times 1050$ .

Fig. 6. *Lepidodendron vasculare*. Transverse section showing the wall of a primary xylem element. The double row of fine threads is seen in the middle of the figure where the section has missed the secondary bars. The piece of underlying wall is at a deeper focus.  $\times 685$ .

Fig. 7. *L. vasculare*. Transverse section showing the primary wall, with a row of dots only on one side, in a differentiating secondary xylem element quite close to the cambium.  $\times 685$ .

Fig. 8. *L. vasculare*. Differentiating secondary xylem elements near the cambium. A row of dots on each side of the primary wall is shown here.  $\times 685$ .

Fig. 9. *Metaclepsydropsis duplex*. Transverse section of petiolar xylem [wall. Pits and pit-membrane.  $\times 650$ .

Fig. 10. Pits and pit-membrane in tangential longitudinal section of the petiolar xylem of *M. duplex*.  $\times 680$ .

Fig. 11. *M. duplex*. Tracheid wall of petiolar xylem. Reticulate type of thickening.  $\times 635$ .

Fig. 12. Transition between scalariform and reticulate thickening in the same element of the petiolar xylem of *M. duplex*.  $\times 635$ .

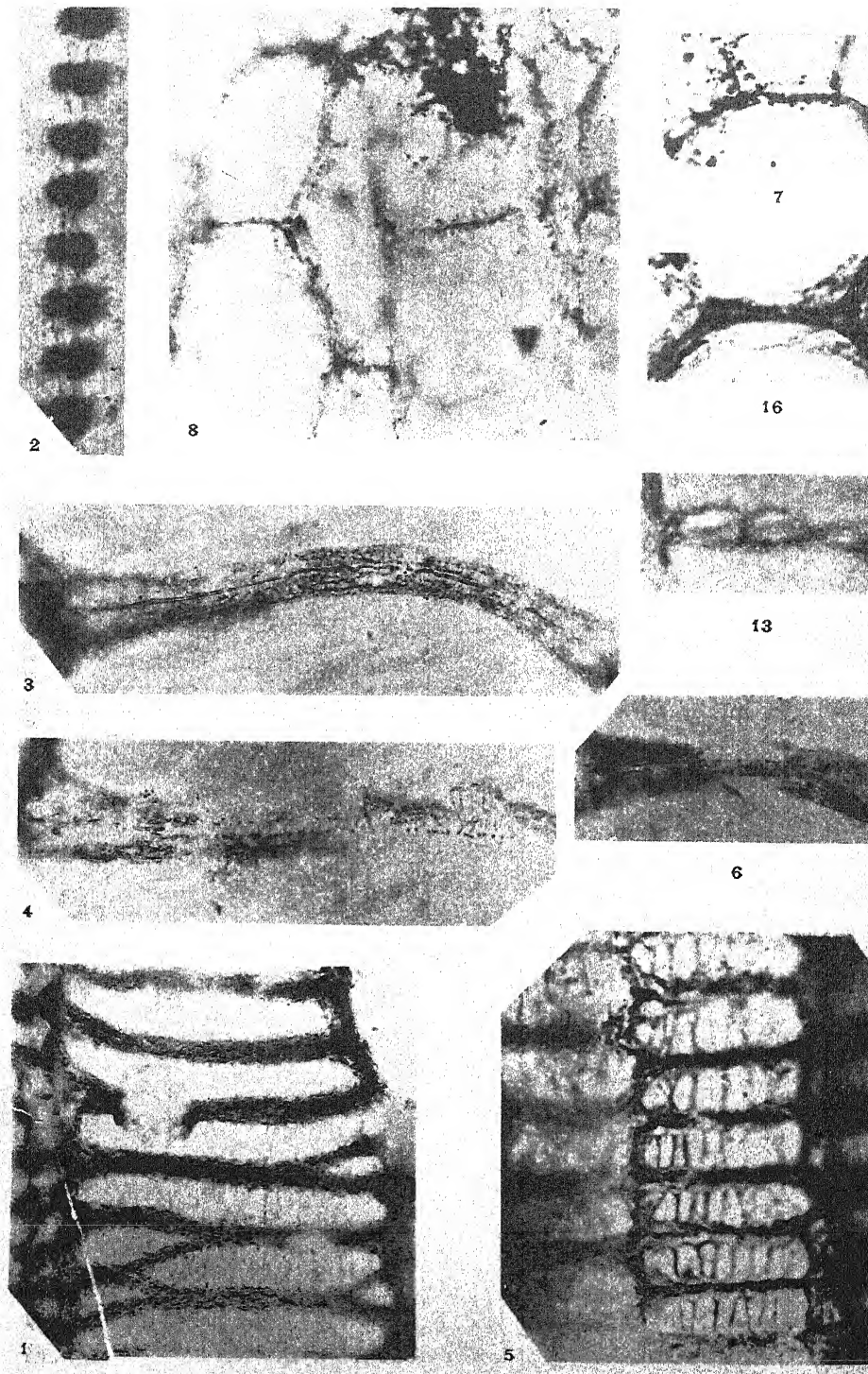
Fig. 13. *Diplolabis Römeri*. Transverse section of tracheid wall with pits and pit-membrane.  $\times 510$ .

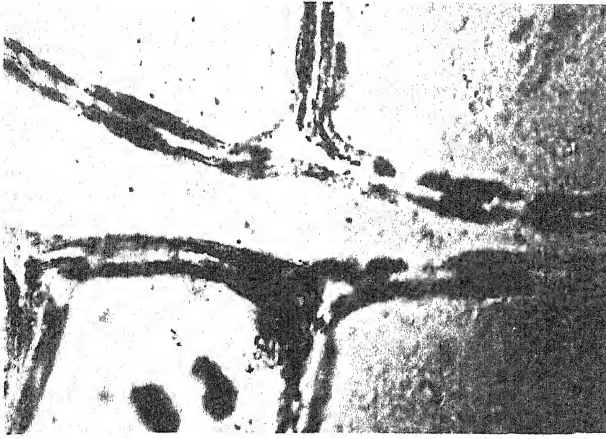
Fig. 14. *Stauropteris burntislandica*. Tangential longitudinal section of the vessel wall, showing the open pits.  $\times 1620$ .

Fig. 15. Face view of a xylem wall of *S. burntislandica*. Bordered scalariform type of pitting and very narrow pit apertures.  $\times 750$ .

Fig. 16. *S. burntislandica*. Transverse section of xylem walls passing through the middle of the bars. The thick primary wall is dark. Secondary walls lighter coloured.  $\times 840$ .

Fig. 17. Transverse section of xylem walls of *S. burntislandica*. The pits are shown, but there is no pit-membrane.  $\times 840$ .

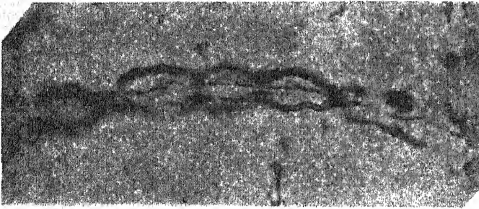




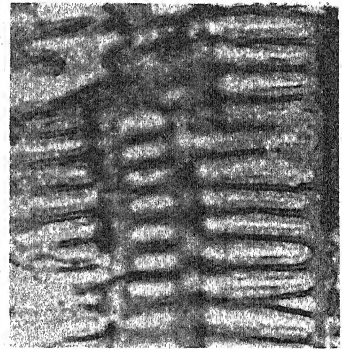
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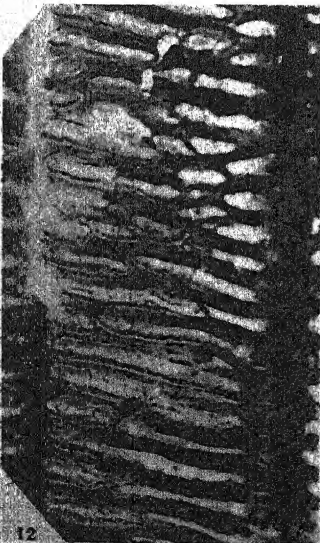
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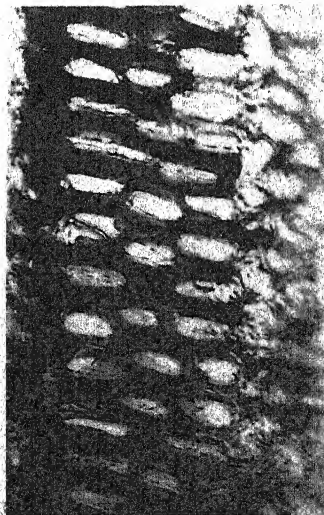
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# Studies in the Genera *Cytosporina*, *Phomopsis*, and *Diaporthe*.

## III. On the Pathogenicity of *Cytosporina ludibunda* and its Saltants.

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With twelve Figures in the Text.

### CONTENTS.

	PAGE
I. INTRODUCTION . . . . .	197
II. PATHOGENICITY OF THE STRAINS OF <i>Cytosporina ludibunda</i> . . . . .	199
III. COMPARISON OF RESULTS OBTAINED IN 1927, 1928, AND 1929 . . . . .	212
IV. PATHOGENICITY IN RELATION TO PEDIGREE . . . . .	214
V. CHANGE IN RATE OF INVASION WITH AGE OF APPLES . . . . .	216
VI. DISCUSSION . . . . .	222
VII. SUMMARY . . . . .	225

### I. INTRODUCTION.

IN the first (14) and second (7) papers of this series an account was given of the occurrence of saltation in some of the genera under investigation. The main characteristics of the saltants, as observed in standard synthetic medium, as well as variations in general morphological characters with change of medium were described. Finally an attempt was made to compare the saltants with certain authentic species of *Phomopsis* and *Diaporthe*. It is proposed now to deal with the attacking power on apple of the strains under investigation, but, for the sake of convenience, only the saltants of *Cytosporina ludibunda* will be considered in the present paper, comparison with *Phomopsis* and *Diaporthe* being left for a later communication.

As mentioned in the preceding paper (7), *Cytosporina ludibunda* from 1920 onwards has been utilized for the work on fungal invasion carried out in this laboratory. In 1926, the year in which saltation was first observed by the writer, Horne ((13) p. 98, Fig. 44), using the parent strain of *Cytosporina ludibunda*, observed certain irregularities in the curves representing

progress of invasion with age of Bramley's Seedling apples. But in the following year, when MK, a saltant of *C. ludibunda*, was used instead of the parent strain, the results were more regular ((12), p. 126, Fig. 47; (13) p. 99, Fig. 45). It was, therefore, thought that the irregularities observed in 1926 were possibly due wholly or in part to dissociation of the parent strain of *C. ludibunda* into saltants of varying attacking power, within the apple tissue.

The present work was therefore undertaken to test the attacking power of the parent and the more stable saltants of *C. ludibunda*, using Bramley's and Worcester apples as hosts. The experiments were carried out for three successive years (1927-9). A list of the strains used is given below, together with reference to the earlier paper (7), where their origin is described.

C Parent strain of *C. ludibunda* ((7) p. 351).

CC Brown saltant with large dark-brown stromata—derived from C ((7) pp. 351-5).

CC<sub>2</sub> Pale orange-yellow non-sporing saltant—derived from CC via CC<sub>1</sub> ((7) p. 353).

CA<sub>1</sub> Grey saltant with few stromata—derived from C through CA ((7) p. 352).

CA<sub>2</sub> Grey non-sporing saltant derived from CA<sub>1</sub> ((7) p. 352).

CA<sub>3</sub> Grey saltant forming numerous stromata derived from CA<sub>1</sub> ((7) p. 353).

CA<sub>4</sub> Black non-sporing saltant derived from CA<sub>3</sub> ((7) p. 353).

MK As mentioned in the previous paper several monohyphal cultures were obtained from the parent strain. Of these, certain cultures which showed a strong resemblance were classed together as MK. This particular group was not studied in detail. Cultures of MK were used for inoculation purposes for a time by Horne and then abandoned in favour of CA<sub>2</sub> (= CE). In standard medium culture MK develops a thin white mycelium, white substratum, and wide zonation. The stromata are large and dark brown and occur sporadically.

For summary of characters of the other strains observed in standard medium cultures see (7) p. 360, Table III.

The estimates of attacking power given in this paper are based on data of the rate of radial advance in apple fruit, calculated by the method formulated by Gregory and Horne (10). The data have been treated, as far as possible, by the method of statistical analysis (9). In experiments where different samples of apples were used for comparisons of attacking power without replicates, differences are regarded as significant only where the odds against the results being fortuitous exceed 100:1.

II. PATHOGENICITY OF THE STRAINS OF *CYTOSPORINA LUDIBUNDA*.

In 1927 two varieties of apple, Bramley's Seedling (from Canterbury) and Worcester Pearmain (from Exning), were used for the inoculation experiment. Four sets in all were available, viz. Early Bramley's, Late Bramley's, Early Worcester, and Late Worcester. Each set was subdivided into samples of twenty, and six strains (C, CC, CC<sub>2</sub>, CA<sub>3</sub>, CA<sub>4</sub>, and MK) were tested. Each sample was inoculated with a given strain, individual apples being inoculated on opposite sides. The inoculated apples were stored at the Low Temperature Station, Cambridge, at 12° C. The dates of inoculation and estimation of different samples are given in tables showing the rate of advance of various strains. The period of storage for the inoculated apples was decided from knowledge of previous growth rate of *C. ludibunda* in apples at that temperature.

During storage a certain amount of loss through natural infection occurred among the inoculated Worcester, hence the number of apples used in each sample for determining radial advance was reduced from twenty to fourteen. The data of mean radial advance in cm. per day for these samples is given in Table I. Owing to the fact that the majority of the apples in samples inoculated with CA<sub>2</sub> and many of those inoculated with CA<sub>4</sub> were completely decayed when examined, the actual value of radial advance should be higher than that given in the table.

TABLE I.

*Worcester Pearmain Apples—Mean Radial Advance in cm. per day.  
12° C. 1927.*

Strain.	Early.		Radial advance.	Radial advance.	Late.	
	Date of inoculation.	Date of estimation.			Date of inoculation.	Date of estimation.
CA <sub>2</sub>	Sept. 14	Dec. 10	> 0.0450	> 0.0680	Oct. 12	Dec. 10
CA <sub>4</sub>	" 14	" 17	> 0.0330	> 0.0460	" 11	" 12
MK	" 16	" 17	0.0187	0.0237	" 12	" 17
CC	" 16	" 17	0.0188	0.0229	" 12	" 19
C	" 15	" 17	0.0152	0.0209	" 13	" 12
CC <sub>2</sub>	" 15	" 19	0.0000	0.0000	" 12	" 19

It is seen at once that the strains vary greatly in attacking power. They fall naturally into three groups: (i) Strong, CA<sub>2</sub> and CA<sub>4</sub>, which are approximately three times as active as the parent strain (C); (ii) Intermediate, C, CC, and MK; and (iii) Weak, CC<sub>2</sub> alone, which proved quite inactive.

In the case of Bramley's Seedling, samples of inoculated apples were reduced to 18 individuals each owing to the loss by natural infection during storage. The data of mean radial advance in cm. per day for all the samples are given in Table II, where the strains are arranged in order of descending values of radial advance as observed for the early Bramley's.

TABLE II.

*Bramley's Seedling Apples. Mean Radial Advance in cm. per day. 12° C. 1927.*

Strain.	Early.			Late.		
	Inoculated.	Estimated.	Radial advance.	Radial advance.	Inoculated.	Estimated.
CA <sub>2</sub>	Oct. 5	Dec. 10	0.0833	0.1100	Nov. 2	Dec. 10
CC	" 7	" 29	0.0109	0.0388	" 3	" 17
MK	" 4	" 13	0.0079	0.0116	Oct. 31	" 7
C	" 7	" 13	0.0058	0.0060	Nov. 2	" 7
CA <sub>4</sub>	" 5	" 17	0.0051	0.0439	" 1	" 17
CC <sub>2</sub>	" 6	" 17	0.0020	0.0016	" 1	" 19

Comparison of Tables I and II shows that the general grouping of the strains with respect to attacking power is affected to a certain extent by variety of apple, since CA<sub>4</sub> found in the 'strong' group in Worcester falls into the 'intermediate' group in the late Bramley's and into the 'weak' group in the early Bramley's.

It should also be noted that the order of strains is not identical in the two sets of Bramley's (early and late), but this is solely due to CA<sub>4</sub> which occupies the fifth and second place in early and late Bramley's respectively.

The significance of the difference between strain and strain as indicated in Table II has been tested, using the standard error of difference of means calculated for each set separately.

The S.E. for all the samples of the early Bramley's set is 0.0026. The S.E. of difference of any two means is therefore  $0.0026 \times \sqrt{2} = 0.0037$ . Similarly, the S.E. for all the samples of the late Bramley's is 0.0065. The S.E. of difference of any two means is 0.0092. Any difference of means which exceeds three times the S.E. of difference (0.0275) is regarded as significant for this set.

The difference between any two means can be readily ascertained from Table III, where in early Bramley's, for example, the difference between the means of C and CC is 0.0051, MK and CC<sub>2</sub> 0.0069, and so on.

The degree of significance indicated by these figures is shown in diagrammatic form in Figs. 1 and 2 for early and late Bramley's respectively.

The following points will be noted in the diagrams: (1) CA<sub>2</sub> differs significantly from all the other strains in both sets of Bramley's. (2) In early Bramley's the remaining strains do not differ significantly. (3) In late Bramley's the strains differ significantly except the following pairs: CA<sub>4</sub> and CC; CC and MK; MK and C; MK and CC<sub>2</sub>; C and CC<sub>2</sub>.

When the strains are arranged in order of attacking power based on the significance of differences, the grouping differs in the early and late sets. In the early set the 'strong' group contains only a single strain CA<sub>2</sub>.

The remaining strains fall into the 'weak' group, there being no intermediates. In the late set the strains again fall into three groups: strong CA<sub>2</sub>; intermediate CA<sub>4</sub> and CC; weak C, CC<sub>2</sub>, and MK.

TABLE III.

*Differences between Mean Radial Advance, cm. per day, of Pairs of Strains.*  
1927.

Bramley's Seedling (Early).					
Strain.	CA <sub>2</sub> .	CC.	MK.	C.	CA <sub>4</sub> .
CC	0.0724				
MK	0.0754	0.0030			
C	0.0775	0.0051	0.0021		
CA <sub>4</sub>	0.0782	0.0058	0.0028	0.0007	
CC <sub>2</sub>	0.0813	0.0089	0.0059	0.0038	0.0031
Bramley's Seedling (Late).					
Strain.	CA <sub>2</sub> .	CA <sub>4</sub> .	CC.	MK.	C.
CA <sub>4</sub>	0.0671				
CC	0.0722	0.0051			
MK	0.0994	0.0323	0.0272		
C	0.1050	0.0379	0.0328	0.0056	
CC <sub>2</sub>	0.1094	0.0423	0.0372	0.0100	0.0044

The strains comprising the 'weak' group include, among others, C the parent strain and the saltant MK. These two do not differ significantly from each other in the experiments described above, although the actual figure for MK is in every case higher than that of C.

Certain supplementary experiments were made to determine whether a real difference between C and MK could be established. In these experiments the strains were tested on the same apples. Each apple was inoculated at four points with C and MK at alternate points. A further experiment was made in a similar way, in which MK (stock culture) was tested against MK, re-isolated from an apple previously inoculated with MK. The experiments carried out are as follows:

Experiment I. Twelve Bramley's Seedling apples inoculated with C and MK on December 8, 1927; kept 12 days. 20° C.

Experiment II. Thirteen Worcester Pearmain apples inoculated with C and MK on November 25, 1927; kept 11 days. 20° C.

Experiment III. Twelve Bramley's Seedling apples inoculated with MK (stock) and MK (re-isolated) on December 8, 1927; kept 12 days. 20° C.

From the data obtained for each experiment the mean radial advance for each strain was calculated. The significance of the difference of these

means has been tested by the method of  $t$  ((9) pp. 104-6). The values of mean radial advance, the values of  $t$ , and the probability ( $P$ ) indicated by the latter are given in Table IV.

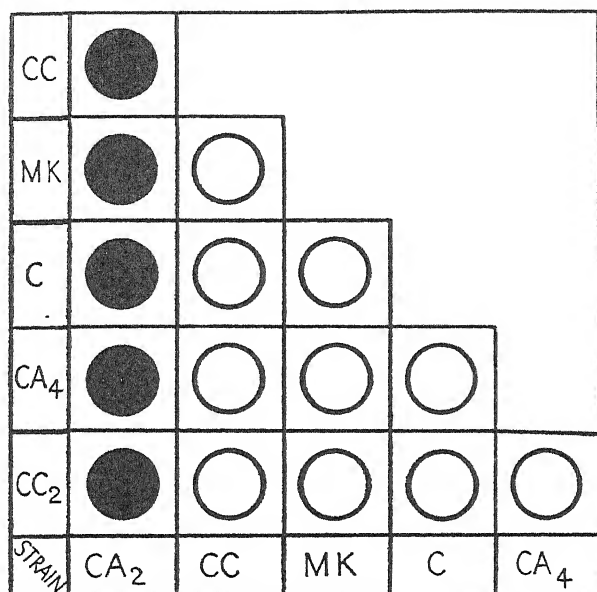





FIG. 1. Diagram showing degree of significance of difference between pairs of strains in early Bramley's Seedling apple, 1927. S.E. = 0.0037. For meaning of signs see below.

 = a difference not exceeding 3SE (not significant)

 = a difference between 3SE and 6SE (significant)

 = a difference between 6SE and 9SE (significant)

 = a difference exceeding 9SE (significant)

Key to Figs. 1-5 and 10-12.

It will be seen from the results of experiments I and II that MK in each case is again more active than C.

Of these results, however, only the first is significant, since out of 100 such random samples only one will by chance give a value of  $t$  exceeding

+3.10 or less than -3.10. The second result is not significant since about 5 out of 100 samples will give a value of  $t$  greater than +2.26 or less than -2.26. The results taken as a whole may indicate a real difference in

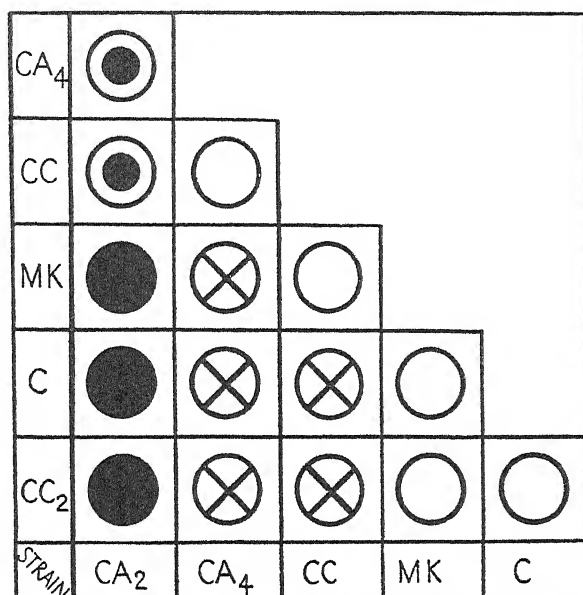


FIG. 2. Diagram showing degree of significance of difference between pairs of strains in late Bramley's Seedling apple, 1927. S.E. = 0.0092. For meaning of signs see p. 202.

attacking power between parent strain and MK. As for Experiment III, the difference in the mean values of the two MK cultures is negligible, indicating that the MK culture had not changed in attacking power whilst in apple tissue.

TABLE IV.

*Attacking Power of MK and C and the Significance of their Difference.*

Experiment.	Mean radial advance, cm. per day.		Value of $t$ .	P.
	MK	C		
I.	0.274	0.131	3.10	0.01
II.	0.383	0.265	2.26	0.05-0.02
	MK. (stock).	MK. (re-isolated).		
III.	0.056	0.058	0.17	0.9-0.8

In 1928 only one variety of apple, Bramley's Seedling (from Canterbury), was used. Two more strains (CA<sub>1</sub> and CA<sub>3</sub>) than in the previous year were included in the test. The experimental method was modified. The

apples were divided into samples of 20 as before, but two strains instead of one were used for each sample, individual apples being inoculated with one strain on one side and with the other strain at a point opposite to the first. Successive samples contained one of the strains used for the preceding sample. Thus for the first sample,  $CA_4$  and  $CA_2$  were used, and for the second sample  $CA_2$  and  $CA_3$ . The complete cycle was as follows:

$CA_4$  and  $CA_2$ ;  $CA_2$  and  $CA_3$ ;  $CA_3$  and  $CA_1$ ;  $CA_1$  and CC; CC and  $CC_2$ ;  $CC_2$  and C; C and MK; MK and  $CA_4$ .

The inoculations were completed between October 10 and October 14 (difference of four days) and the estimations between November 2 and November 28 (difference of twenty-six days). The longest time that elapsed between the estimation of the first and second samples inoculated with the same strain was fifteen days in the case of  $CA_4$ . The experiment was carried out at laboratory temperature ( $18^\circ\text{C}.$ – $20^\circ\text{C}.$ ).

The data of mean radial advance in cm. per day, obtained for each strain, on the basis of 18 half-apples in a sample is given in Table V.

TABLE V.

*Mean Radial Advance in cm. per day ( $18^\circ\text{C}.$ – $20^\circ\text{C}.$ ) Bramley's Seedling Apple. 1928.*

Strain.	Mean radial advance, cm. per day.		
	Sample I.	Sample II.	Mean.
$CA_2$	0.0800	0.0642	0.0721
$CA_1$	0.0491	0.0655	0.0573
$CA_3$	0.0523	0.0419	0.0471
MK	0.0266	0.0396	0.0331
CC	0.0128	0.0254	0.0191
$CA_4$	0.0087	0.0274	0.0180
C	0.0148	0.0194	0.0171
$CC_2$	0.0028	0.0017	0.0023

It is seen from Table V that again the strains differ from one another, the differences between extremes being considerable ( $CC_2$ , 0.0028;  $CA_2$ , 0.0800). Further, the values from the first and second samples of any one strain are not the same. For example,  $CA_3$  has a mean radial advance of 0.0523 cm. in the first and 0.0419 cm. in the second sample, and with  $CA_4$  the mean radial advance is 0.0087 and 0.0274 cm. per day in the first and second samples respectively.

The significance of these observed differences among the strains has been tested by the analysis of variance. Owing to the fact that these strains have always been associated in pairs, there is a correlation between values of the radial advances of pairs of strains tested. By taking mean values of grouped figures obtained for replicates for each strain, the values obtained are not strictly speaking random values. To test whether this has introduced an appreciable error in the estimates of radial advance, the



variance represented by the differences between the grouped values has been calculated and compared with the remainder error. The figures have been further analysed for the significance of interaction, which in this case represents the variance of differences of the values of radial advance for each fungus as estimated on the two groups of 18 half-apples; that is, where strain  $CA_2$  is associated with  $CA_4$  as against  $CA_2$  associated with  $CA_3$ ; the strain CC associated with  $CA_1$  as against CC associated with  $CC_2$ , and so on. The full analysis is given in Table VI.

Since there are 16 samples, each consisting of 18 half-apples, there will be 287 degrees of freedom in all; made up of 7 degrees of freedom for strains; 1 for groups mentioned above; 7 for interaction, and 272 for error (variance within samples).

TABLE VI.

*Analysis of Variance. Attacking Power of Strains on Bramley's Seedling Apple. 1928.*

	Degrees of freedom.	Sum of squares.	Variance.	S.D.	Log S.D.	Value of $z$ .
Strains	7	0.141520	0.02022	0.14200	-1.95192	+1.68744
Grouping	1	0.001591	0.00159	0.03988	-3.22186	+0.41750
Interaction	7	0.008072	0.00115	0.03391	-3.38404	+0.25532
Error (within sample)	272	0.187650	0.00069	0.02627	-3.63936	

For  $n_1 = 7$  and  $n_2 = 272$  1% point of value of  $z$  is about 0.5152.

„  $n_1 = 1$  „  $n_2 = 272$  5% „ „ „ 0.6729.

„  $n_1 = 7$  „  $n_2 = 272$  5% „ „ „ 0.3706.

The value of  $z$  for strains is +1.687. This value is more than three times the 1 per cent. point, indicating that, taken as a whole, the strains differ significantly.

The value of  $z$  for grouping is +0.4175, a value less than the 5 per cent. point, showing that the differences due to grouping is within the experimental error.

The value of  $z$  for interaction is +0.2553, which is also below the 5 per cent. point. Interaction, therefore, is not significant.

It has been shown by analysis of variance that the strains differ in attacking power. The precise nature of this difference will now be considered, using the average of the mean value of two samples (36 half-apples as given in the last column of Table V).

The standard deviation for the whole experiment is 0.02627. The standard error for groups of 36 half-apples is  $\frac{0.02627}{\sqrt{36}} = 0.0044$ . The standard error of difference between any two means is

$$(0.0044 \times \sqrt{2}) = 0.0062.$$

The difference between mean values of any given pair of strains is given in Table VII.

TABLE VII.

*Differences between Mean Radial Advance (cm. per day) of Pairs of Strains.  
Bramley's Seedling Apple. 1928.*

Strain.	CA <sub>2</sub>	CA <sub>1</sub>	CA <sub>3</sub>	MK	CC	CA <sub>4</sub>	C
CA <sub>1</sub>	0.0148						
CA <sub>3</sub>	0.0250	0.0102					
MK	0.0390	0.0242	0.0140				
CC	0.0530	0.0382	0.0280	0.0140			
CA <sub>4</sub>	0.0544	0.0396	0.0294	0.0154	0.0014		
C	0.0550	0.0402	0.0300	0.0160	0.0020	0.0006	
CC <sub>2</sub>	0.0698	0.0550	0.0448	0.0308	0.0168	0.0154	0.0148

The degree of significance indicated by the above figures is represented diagrammatically in Fig. 3.

It will be seen from the diagram that CA<sub>2</sub> differs significantly from all but CA<sub>1</sub>; CA<sub>3</sub> from all but CA<sub>1</sub> and MK; CA<sub>1</sub> from all but CA<sub>2</sub> and CA<sub>3</sub>; MK differs significantly from CA<sub>2</sub>, CA<sub>1</sub>, and CC<sub>2</sub> only. The strains CC, CA<sub>4</sub>, C, and CC<sub>2</sub> do not differ from each other.

It has been shown by the analysis of variance that the differences in radial advance due to grouping are not significant. It does not necessarily follow that differences between every pair of values for individual strains should be without significance. It will be seen from Table VIII that the radial advance of CA<sub>4</sub> in sample II is about three times as great as that shown in sample I. In order to test whether any significance should be attached to this difference a further analysis was made. In this analysis the effect of samples on radial advance was determined for individual strains by calculating the standard error of difference of means ( $\frac{S\sqrt{2}}{\sqrt{n}}$ ) for each pair of values.

The result of this analysis is given in Table VIII, where the differences of mean values of radial advance of the two samples are shown for each strain in the fourth column. The fifth column gives the S.E. of difference for the samples for purposes of comparison with column 4.

It is seen that for CA<sub>4</sub> the difference between the mean values of the two samples is more than five times its standard error of difference, and for CC it is three times as great. If three times the S.E. is taken as the minimum for significance, the samples in their reaction to CA<sub>4</sub> can be regarded as significantly different, while for CC and MK, where the difference is almost three times S.E., as also for the remaining strains, the differences due to sampling are not significant.

The difference between samples of apples as brought out by their

behaviour towards  $CA_4$  is probably an effect due to age of apples, since sample II with  $CA_4$  was inoculated later than sample I and was kept a

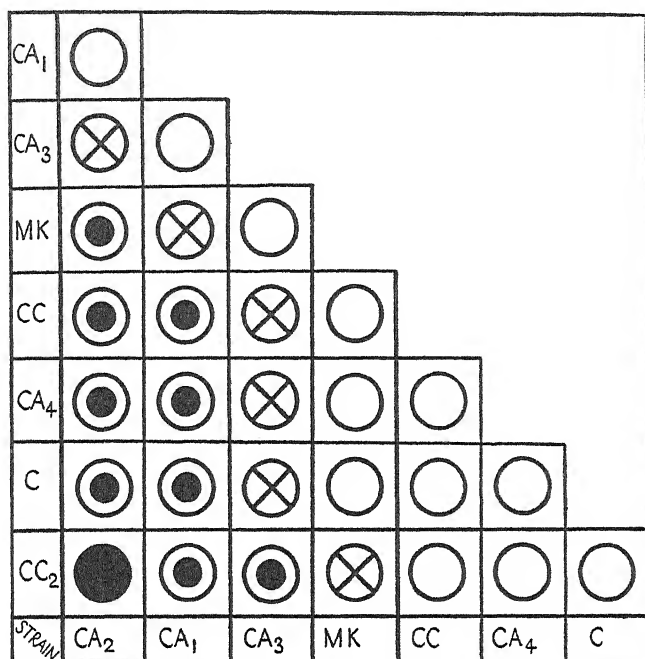


FIG. 3. Diagram showing degree of significance of difference between pairs of strains in Bramley's Seedling apple, 1928. S.E. = 0.0062. For meaning of signs see p. 202.

TABLE VIII.

*Significance of the Effect of Sampling on Strains. 1928.*

Mean radial advance, cm. per day.

Strain.	Sample I (R.A. <sub>1</sub> )	Sample II (R.A. <sub>2</sub> )	Difference of means.	S.E. of difference.
CA <sub>4</sub>	0.0087	0.0274	0.0187	0.0034
CC	0.0128	0.0254	0.0126	0.0046
MK	0.0266	0.0396	0.0130	0.0054
CA <sub>1</sub>	0.0491	0.0655	0.0164	0.0112
CC	0.0148	0.0194	0.0046	0.0046
CA <sub>2</sub>	0.0800	0.0642	0.0158	0.0160
CC <sub>2</sub>	0.0028	0.0019	0.0011	0.0012
CA <sub>3</sub>	0.0523	0.0419	0.0104	0.0122

longer time in storage before estimating the amount of decayed tissue. The time-effect indicated here will be specially dealt with in a later section.

When arranged in order of attacking power, the strains show a general correspondence with the order obtained for early Bramley's in 1927. The additional strains  $CA_1$  and  $CA_3$  may be grouped with  $CA_2$ .

In 1929 the tests were carried out on Bramley's Seedling (from Cambridge) and Worcester Pearmain apples (from Cambridge), using the method adopted in 1927. The apples were inoculated on both sides with the same strain and the strains tested were those used in 1927. Since only a limited number of apples was available the samples used consisted of only ten individuals. After inoculation the apples were kept at room temperature ( $18^{\circ}\text{C.}$ – $20^{\circ}\text{C.}$ ). The rate of radial advance in cm. per day for each strain was calculated from the raw data obtained.

The full results are given in Table IX, where the strains are arranged in order of their attacking power in Bramley's. The order in Worcester is given in Roman numerals in the last column.

TABLE IX.

*Bramley's Seedling and Worcester Pearmain Apples. Mean Radial Advance of Strains in cm. per day ( $18^{\circ}\text{C.}$ – $20^{\circ}\text{C.}$ ). 1929.*

Mean radial advance, cm. per day.			
Strain.	Bramley's.	Worcester.	Order for Worcester.
CA <sub>2</sub>	0.1176	0.0743	II
MK	0.0400	0.0581	III
CC	0.0391	0.0158	VI
CA <sub>4</sub>	0.0360	0.0966	I
C	0.0273	0.0176	V
CC <sub>2</sub>	0.0057	0.0192	IV

It is seen from Table IX that, as in previous years, the strains differ greatly in their attacking power, and that in Bramley's the grouping corresponds to that obtained for the early Bramley's of 1927; e.g. 'strong' CA<sub>2</sub>; 'intermediate' MK, CC, CA<sub>4</sub>, and C; 'weak' CC<sub>2</sub>. In Worcester the grouping is different. The 'strong' group comprises CA<sub>4</sub>, CA<sub>2</sub>, and MK; the 'intermediate' CC, C, and CC<sub>3</sub>. The 'weak' group is not represented.

The significance of the observed differences for strains, variety, and the differential effect of variety on strains (interaction) have been tested by analysis of variance, but the considerations of space do not allow of the inclusion of details of the analysis.

It was found that the value of  $z$  for strains is +1.662, which is three times the value of the 1 per cent. point (0.5522), indicating that the strains differ significantly in attacking power. The value of  $z$  for varieties is -0.5904, showing that the differences due to varieties when results for all the strains in each variety are considered together are well within the experimental error. The value of  $z$  for interactions is +1.1195, which is twice the value of the 1 per cent. point (0.6729), indicating a significant differential effect of variety on strain.

The difference between strains already brought out by analysis will now be studied in greater detail, taking into consideration the results obtained from Bramley's and Worcester separately.

Since the difference due to varieties, when all the strains are considered together, is not significant, the standard deviation (0.0267) for the entire experiment can be utilized for both Bramley's and Worcester. The standard error of difference of means will therefore be  $\frac{0.0267 \times \sqrt{2}}{\sqrt{10}} = 0.012$ .

Differences of means for paired strains are presented separately for Bramley's and Worcester in Table X.

TABLE X.

*Differences between Mean Radial Advance (cm. per day) of Pairs of Strains. 1929.*

Bramley's Seedling Apple.					
Strain.	CA <sub>2</sub> .	MK.	CC.	CA <sub>4</sub> .	C.
MK	0.0776				
CC	0.0785	0.0009			
CA <sub>4</sub>	0.0816	0.0040	0.0031		
C	0.0913	0.0127	0.0118	0.0087	
CC <sub>2</sub>	0.1119	0.0343	0.0334	0.0303	0.0216
Worcester Pearmain Apple.					
Strain.	CA <sub>4</sub> .	CA <sub>2</sub> .	MK.	CC <sub>2</sub> .	C.
CA <sub>2</sub>	0.0223				
MK	0.0385	0.0162			
CC <sub>2</sub>	0.0774	0.0551	0.0389		
C	0.0790	0.0567	0.0405	0.0016	
CC	0.0808	0.0587	0.0423	0.0034	0.0018

The degree of significance indicated by the above figures is represented diagrammatically in Figs. 4 and 5.

It is seen from Fig. 4 that in Bramley's CA<sub>2</sub> is significantly different from all the other strains. The remaining strains do not differ from one another.

In Worcester (Fig. 5) the following pairs of strains show no significant difference: CA<sub>4</sub> and CA<sub>2</sub>; CA<sub>2</sub> and MK; CC<sub>2</sub> and C; CC<sub>2</sub> and CC; C and CC. The rest differ significantly from each other.

That the varietal differences in the composition of apples act differentially on the strains has been brought out by the analysis of the interaction between strain and variety. In Table XI the differences found between mean values of radial advance for individual strains in two varieties are given together with the standard error of difference of means in each case. Differences of mean values exceeding three times the S.E. of difference of means are considered significant.

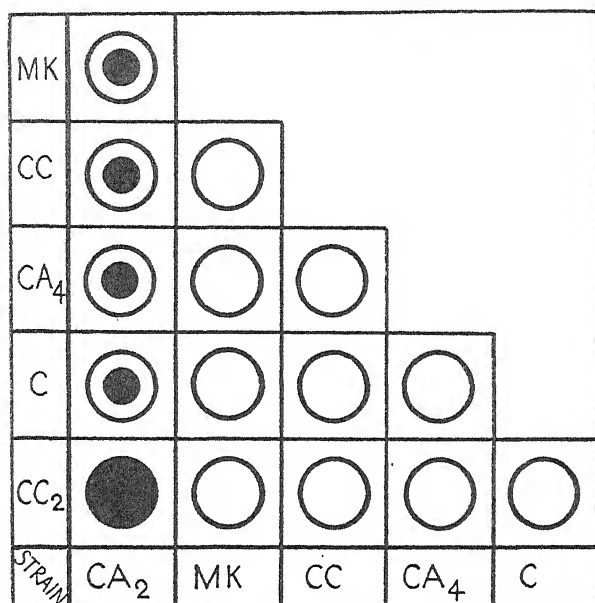


FIG. 4. Diagram showing degree of significance of difference between pairs of strains in Bramley's Seedling apple, 1929. S.E. = 0.0120. For meaning of signs see p. 202.

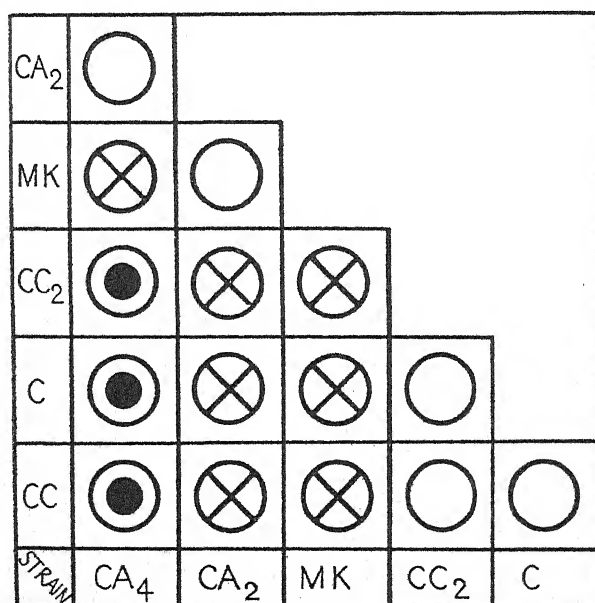


FIG. 5. Diagram showing degree of significance of difference between pairs of strains in Worcester Pearmain apple, 1929. S.E. = 0.0120. For meaning of signs see p. 202.

TABLE XI.

*Significance of the Differential Effect of Varieties on Strains. 1929.*

Mean radial advance, cm. per day.

Strain.	Bramley's.	Worcester.	Difference of means.	S.E. of difference.
CA <sub>4</sub>	0.0360	0.0966	0.0606	0.0101
CC <sub>2</sub>	0.0057	0.0192	0.0135	0.0045
CC	0.0391	0.0158	0.0233	0.0087
MK	0.0400	0.0581	0.0181	0.0080
CA <sub>2</sub>	0.1176	0.0743	0.0433	0.0226
C	0.0273	0.0176	0.0097	0.0089

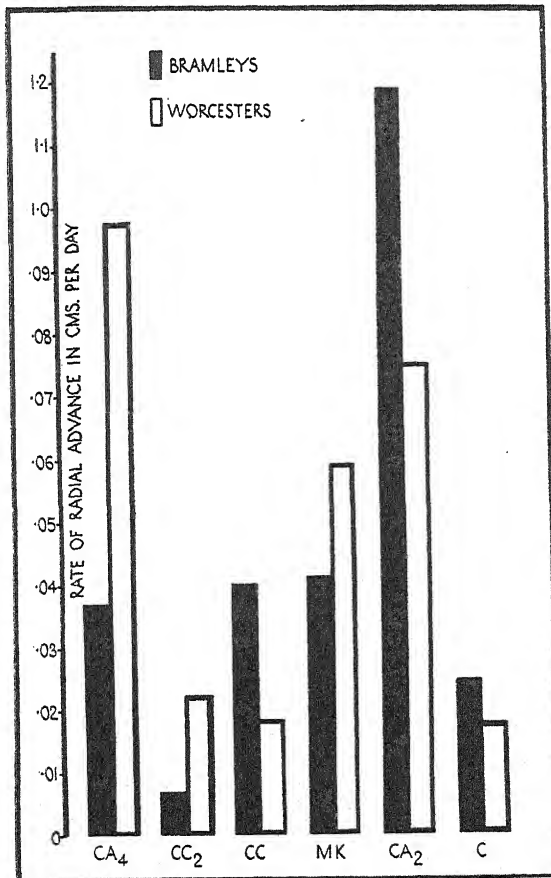


FIG. 6. Diagram showing rate of radial advance of strains of *Cytosporina ludibunda* in Bramley's Seedling and Worcester Pearmain apple, 1929.

It is seen from the table that the difference of means for CA<sub>4</sub> is six times the S.E. and is therefore markedly significant. With CC<sub>2</sub> the

difference is barely significant. The differences shown by the remaining strains are within the limits of experimental error.

These differential activities of the strains in two varieties are represented diagrammatically in Fig. 6, where the height in each column represents the radial advance in cm. per day.

It is seen that CA<sub>4</sub>, the only strain significantly different, attacks Worcester more strongly than Bramley's, the actual rate in Worcester being three times that in the other variety. This confirms the observation of 1927, when CA<sub>4</sub> was found to be more active in Worcester than in Bramley's.

Among the strains (CA<sub>2</sub>, C, CC, CC<sub>2</sub>, and MK) which do not differ significantly in either variety, the values of radial advances recorded for MK and CC<sub>2</sub> are higher for Worcester than for Bramley's, but for CC, CA<sub>2</sub> and C the reverse relation holds.

### III. COMPARISON OF RESULTS OBTAINED IN 1927, 1928, AND 1929.

Since the experimental conditions of temperatures and the source of apples in the first year (1927) differed from those of subsequent years, the results of all the three years could not be analysed together. It was, however, possible to analyse the results of 1928 and 1929 for Bramley's Seedling apple, since in both the years the experiments were carried out under similar conditions, apples being obtained from the same locality and inoculated with the same strains.

The samples in 1928 consisted of 18 individuals each, instead of 10 as in 1929. For analysis of variance a fresh mean was calculated for the former on the basis of ten apples in a sample. The means are given in Table XII.

TABLE XII.

*Mean Radial Advance in cm. per day. 1928 and 1929.  
(On the Basis of ten Apples per Sample.)*

Strain.	Bramley's Seedling. Mean radial advance, cm. per day.	
	1928.	1929.
CA <sub>2</sub>	0.0738	0.1176
MK	0.0340	0.0400
CC	0.0193	0.0391
CA <sub>4</sub>	0.0184	0.0360
C	0.0124	0.0273
CC <sub>2</sub>	0.0027	0.0057

These differences in radial advance observed in two years were tested by analysis of variance for strains, year, and for differential effect of year on strains (interaction).

The analysis showed that the value of  $z$  for strains is +2.1868, while the



value of the 1 per cent. point is 0.6028. The difference among the strains is therefore to be considered very significant. The value of  $z$  for *year* is +1.8022, about twice the value of the 1 per cent. point (0.9784), indicating that taken as a whole, the rate of attack of the strains in 1928 was significantly, different from that observed in 1929. The value of  $z$  for *interaction* (+1.2203) is twice the value of the 1 per cent. point (0.6028) and is therefore clearly significant. Interaction in this case will mean the complex effect of such factors as (1) Seasonal differences in the composition of apples: (2) Age of apple at the time of inoculation; (3) Possible changes in the attacking power of strains.

The effect of these combined factors on individual strains is further elucidated by the following table, where the differences between values of radial advance in Bramley's of 1928 and of 1929 are given for each strain, together with their S.E. of difference. The former should be three times the latter to be considered significantly different.

TABLE XIII.

*Significance of the Differential Effect on the Strains of the Bramley's of 1928 and of 1929.*

Strain.	Mean radial advance, cm. per day.		Difference of means.	S.E. of difference.
	1928	1929		
CA <sub>4</sub>	0.0184	0.0360	0.0176	0.0055
CC <sub>2</sub>	0.0027	0.0057	0.0030	0.0010
CA <sub>2</sub>	0.0738	0.1176	0.0438	0.0205
CC	0.0193	0.0391	0.0198	0.0093
C	0.0124	0.0273	0.0149	0.0090
MK	0.0340	0.0400	0.0060	0.0085

It is seen that only CA<sub>4</sub> and CC<sub>2</sub> differ significantly in the two years.

Reference to Table XIV will show that when arranged in order of the values of radial advance in Bramley's Seedling, the strains of *C. ludibunda* fall into series which are identical in 1928 and 1929. Although the experiments in 1927 were carried out at the lower temperature of 12° C., using early and late Bramley's obtained from a different locality (Canterbury), the order of the strains approximates to that found for 1928 and 1929.

The order of the strains in Bramley's for the three years is given in Table XIV. The strains bracketed do not differ significantly from each other.

It is seen that in early Bramley's such variations in order as do occur cannot be considered significant since they involve only those strains which in the particular experiment show no significant difference from each other. In the late Bramley's of 1927 and the Bramley's of 1928, CA<sub>4</sub> occupies the second and fourth places respectively, while MK occupies the fourth and

the second. The difference in the order in this case must be considered significant, since the strains involved are significantly different from each other.

TABLE XIV.

*Strains arranged in Descending Order of Attacking Power on Bramley's. 1927-9.*

1929	1928	1927	
		Early.	Late.
CA <sub>2</sub>	CA <sub>2</sub>	CA <sub>2</sub>	CA <sub>2</sub>
{ MK	{ MK	{ CC	{ CA <sub>4</sub>
{ CC	{ CC	{ MK	{ CC
{ CA <sub>4</sub>	{ CA <sub>4</sub>	{ C	{ MK
{ C	{ C	{ CA <sub>4</sub>	{ C
{ CC <sub>2</sub>	{ CC <sub>2</sub>	{ CC <sub>2</sub>	{ CC <sub>2</sub>

The order of the strains varies with variety of apples. This is evident from the experiment carried out in 1929, in which the same strains were compared, using Bramley's Seedling and Worcester Pearmain from the same locality under similar experimental conditions. The order of attacking power in the two varieties is given in Table XV. The bracketed strains are not significantly different.

TABLE XV.

*Strains arranged in Descending Order of their Attacking Power on Bramley's and Worcester. 1929.*

Bramley's.	Worcester.
CA <sub>2</sub>	{ CA <sub>4</sub>
{ MK	{ CA <sub>2</sub>
{ CC	{ MK
{ CA <sub>4</sub>	{ CC <sub>2</sub>
{ C	{ C
{ CC <sub>2</sub>	{ CC

The most pronounced effect of variety is seen in the change of position of strain CA<sub>4</sub>, which occupies the first place in Worcester and only the fourth in Bramley's. Further CC<sub>2</sub>, a strain consistently the weakest in Bramley's, proved more active in Worcester and occupies the fourth place there. The fairly active strain CC proves the weakest in Worcester.

#### IV. PATHOGENICITY IN RELATION TO PEDIGREE.

It was apparent from the result of the experiment carried out in 1927 that at least one of the most active saltants was derived from a weaker parent. The experimental work of 1928 was designed to test attacking power in relation to pedigree. For this purpose the strain C, from which

all the saltants were obtained, was used together with as many descendants from the strain as were available at the time.

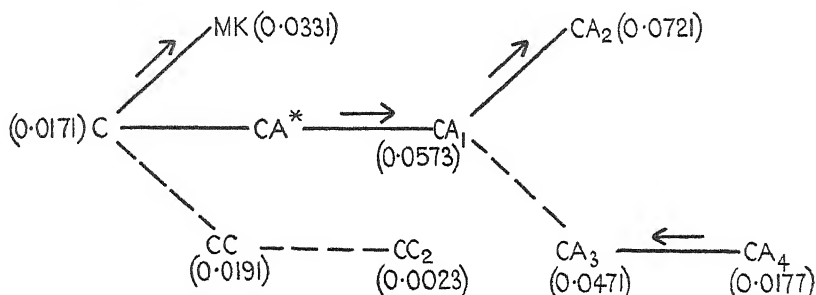


FIG. 7. Diagrammatic representation of the descent of strains of *C. ludibunda*. The mean value of radial advance (cm. per day) for each strain is given within brackets.

\* Radial advance not determined.

— Denotes significance of difference between saltant and immediate parent.

- - - Denotes no significance of difference between saltant and immediate parent.

The arrow (→) points towards the more virulent strain.

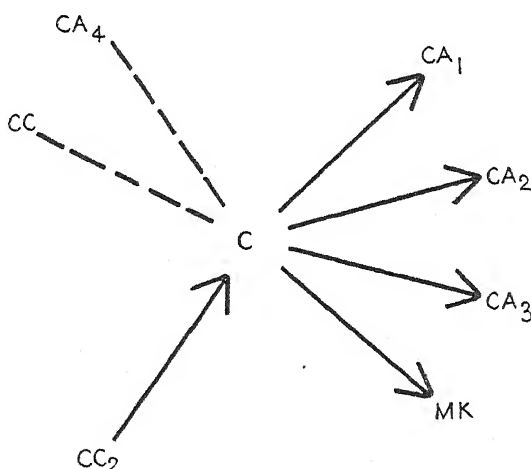


FIG. 8. *Cytosporina ludibunda*. Diagram indicating relative pathogenicity of the saltants compared with that of the parent C.

— Denotes significance of difference between parent and saltant.

- - - Denotes no significance of difference between parent and saltant.

The arrow (→) Points towards the more virulent strain.

The lines of descent of the strains and the mean radial advance in cm. per day in Bramley's Seedling apples for each strain is given in Fig. 7.

It is seen that the original parent, C, has given rise to a saltant MK, which is significantly more active, and to another saltant CC, from which it does not differ significantly. CC in its turn has possibly given rise to a slightly less active strain, CC<sub>2</sub>, the difference, however, is not significant.

The saltant  $CA_1$ , derived from C via CA, is outstandingly more active than C. This  $CA_1$  has given rise to two strains, (1)  $CA_2$  which is more active, and (2)  $CA_3$  which is slightly, though not significantly, less active.

$CA_3$  has given rise to a significantly less active strain  $CA_4$ .

When the pathogenicity of the saltants is compared with that of the original strain C (Fig. 8) it is found that the saltants  $CA_1$ ,  $CA_2$ ,  $CA_3$ , and MK are more virulent than C. Saltants  $CA_4$  and CC do not differ significantly from C in virulence, and  $CC_2$  is less virulent than C.

It should be noted that this relation between the parent strains and the saltants in regard to pathogenicity is liable to variation with alteration in the experimental factors and also with the variety of apples used. Under all conditions, however, saltants have been found which are significantly more active than the original parent C.

#### V. CHANGE IN RATE OF INVASION WITH AGE OF APPLES.

It has already been mentioned (p. 199) that the inoculation experiments of 1927 were carried out on apples gathered on two different occasions, designated early and late. Since the other experimental factors were the same the differences in the rate of invasion shown by strains in these two sets of apples should indicate the effect of age of apple on rate of invasion and, inversely, on resistance to invasion. The values of mean radial advance in cm. per day for each strain in both sets have already been given (Table II). The results are also presented graphically in Fig. 9.

It will be seen that for all the strains except  $CC_2$  the values are greater in the late set than in the early set. The strains are, however, differentially affected by the age of the apple. For example, the radial advance of  $CA_4$  has changed from 0.0051 cm. per day for the early set to 0.0439 cm. per day for the late, while that of C from 0.0058 cm. per day to 0.0060 cm. per day. It will also be noted that CC, which is more active than  $CA_4$  in the early set, is less active than the same in the late set.

The significance of the observed differences between mean values of radial advance in early and late sets of Bramley's has been tested by the analysis of variance, both for the effect of age and the differential effect of age on strains (interaction).

It was found from analysis that the value of  $z$  for age is +1.7454, about twice that of the 1 per cent. (0.9558), indicating that age of fruit has a real effect on the rate of invasion.

The value of  $z$  for interaction (+1.2670) is much higher than that of the 1 per cent. (0.5674), indicating a differential effect of age of apple on attacking power of strains.

This effect of age of apple on individual strains has been ascertained

separately by comparing the difference of mean values in the early and late sets for each strain with its S.E. of difference (Table XVI).

It will be observed that the difference of means for CC is about ten times and for CA<sub>4</sub> about four times their respective S.E. of difference. These

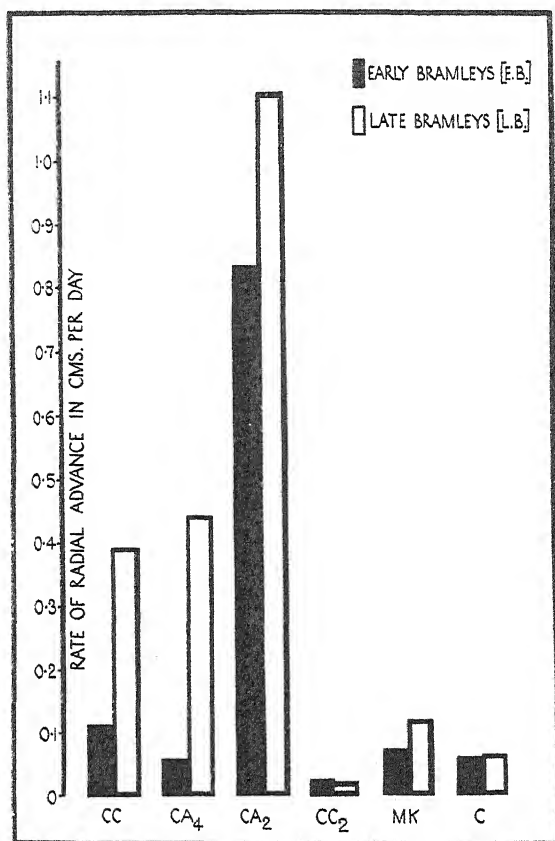


FIG. 9. Diagram showing rate of radial advance of strain *C. ludibunda* in early and late Bramley's Seedling apple at 12°C., 1927.

strains are therefore significantly different in the early and late sets. With CA<sub>2</sub>, however, the difference falls just short of twice the S.E. of difference, therefore the chance that CA<sub>2</sub> is different in the late and early sets is less than 20:1. The difference shown by the other strains are still less significant.

It is evident from the above that the age of Bramley's apple has a definite influence on the attacking power of some strains, but may have little effect upon others. The late Bramley's are more susceptible than the early ones.

TABLE XVI.

*Significance of the Differential Effect on Strains of Age of Bramley's Seedling (1927).*

Mean radial advance, cm. per day.				
Strain.	Early.	Late.	Difference of means.	S.E. of difference.
CC	0.0109	0.0388	0.0279	0.0028
CA <sub>4</sub>	0.0051	0.0439	0.0388	0.0090
CA <sub>2</sub>	0.0833	0.1100	0.0267	0.0138
CC <sub>2</sub>	0.0020	0.0016	0.0004	0.0007
MK	0.0079	0.0116	0.0037	0.0091
C	0.0058	0.0060	0.0002	0.0011

Additional data for three of the strains, CA<sub>2</sub>, CA<sub>4</sub>, and MK, have been placed at the author's disposal by Dr. A. S. Horne. The data in question were obtained in 1929 with Bramley's Seedling apple gathered all at one time. Sets of sixty apples, twenty for each strain, were inoculated at weekly intervals and the inoculated apples were kept at 20° C. Estimates of decayed tissue were made periodically as shown in Table XVII. In the same table is given the data of mean radial advance in cm. per day based on sixteen apples in a sample for CA<sub>2</sub> and CA<sub>4</sub> and ten apples in a sample for MK.

TABLE XVII.

*Mean Radial Advance in cm. per day for CA<sub>2</sub>, CA<sub>4</sub>, and MK. Bramley's Seedling. 20° C. 1929.*

Samples.	Inoculated.	No. of days in store.	Radial advance, cm. per day.		
			CA <sub>2</sub>	CA <sub>4</sub>	MK
A	Oct. 22	21	0.0601	0.0039	0.0126
B	" 30	20	0.0868	0.0068	0.0172
C	Nov. 6	20	0.1340	0.0211	0.0242
D	" 20	20	0.1853	0.0254	0.0444
E	" 27	18	0.3090	0.0564	0.0713
F	Dec. 4	14	0.2512	0.0563	
G	" 11	15	0.3410		

There is a gradual increase in the rate of attack with increasing age of apples, except in sample F. The significance of this observed effect of age on the rate of attack has been tested by analysis of variance, taking each strain separately.

The results in abridged form are given in Table XVIII.

1. CA<sub>2</sub>. It will be seen from Table XVIII that the value of  $z$  for age is three times that of the 1 per cent. point, indicating that the effect of age of apple on the attacking power of CA<sub>2</sub> is significant.

The difference between mean values of radial advance for any two

samples are given in Table XIX, and the degree of significance shown by these differences is diagrammatically represented in Fig. 10.

TABLE XVIII.

*Analysis of Variance of Change with 'Age' of Susceptibility of Bramley's to Attack by CA<sub>2</sub>, CA<sub>4</sub>, and MK.*

Strain.	<i>z</i>	1 % point.	S.E. of difference of mean radial advance.
CA <sub>2</sub>	+1.628	0.5152	0.0300
CA <sub>4</sub>	+1.657	0.5522	0.0062
MK	+1.413	0.6472	0.0083

TABLE XIX.

*Differences between Means of Radial Advance in Pairs of Samples of Bramley's Apples of Different Age. Strain CA<sub>2</sub>.*

Sample.	A.	B.	C.	D.	E.	F.
B	0.0267					
C	0.0739	0.0472				
D	0.1252	0.0985	0.0513			
E	0.2489	0.2222	0.1750	0.1237		
F	0.1911	0.1644	0.1172	0.0659	0.0578	
G.	0.2809	0.2542	0.2070	0.1557	0.0320	0.0898

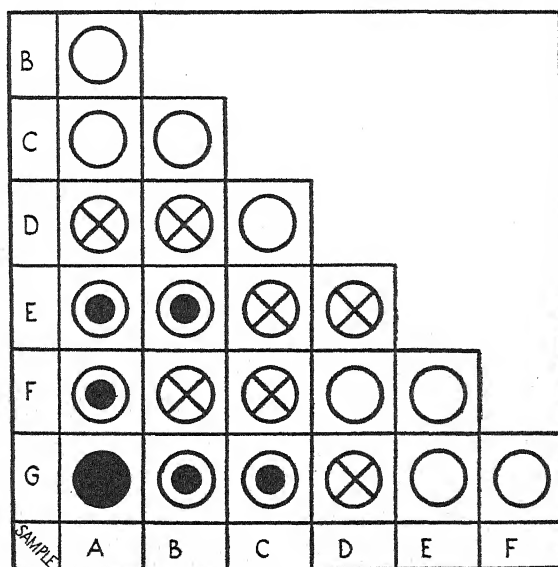


FIG. 10. Diagram showing degree of significance with age between pairs of samples of Bramley's Seedling apple inoculated with strain CA<sub>2</sub>, 1929. S.E. = 0.0300. For meaning of signs see p. 202.

It will be observed that there is no significant difference in successive pairs of samples except in the case of samples D and E. The difference becomes significant as the difference between age of samples increases.

2.  $CA_4$ . The value of  $z$  for age is three times the value of the 1 per cent. point (Table XVIII). Hence the effect of age of apple on the attacking power of  $CA_4$  is significant.

The significance of difference between pairs of samples is analysed below. The S.E. of difference here equals 0.0062.

TABLE XX.

*Differences between Mean Radial Advance in Pairs of Samples of Bramley's Apples of Different Age. Strain  $CA_4$ .*

Sample.	A	B	C	D	E
B	0.0029				
C	0.0172	0.0143			
D	0.0215	0.0186	0.0043		
E	0.0525	0.0496	0.0353	0.0310	
F	0.0524	0.0495	0.0352	0.0309	0.0001

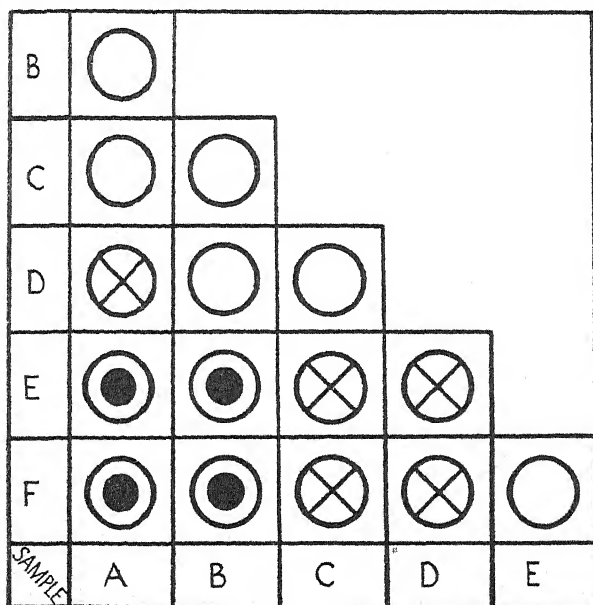


FIG. 11. Diagram showing degree of significance with age between pairs of samples of Bramley's Seedling apple, inoculated with strain  $CA_4$ , 1929. S.E. = 0.0062. For meaning of signs see p. 202.

As with  $CA_2$ , consecutive samples inoculated with  $CA_4$  do not differ significantly, with the exception of the pair D and E.

3. MK. Table XVIII shows that the value of  $z$  for 'age' is three



times that for the 1 per cent. point, and therefore the effect of age of apple on the attacking power of MK is significant.

The significance of the difference between pairs of samples is given below.

The S.E. of difference of mean radial advance is 0.0083.

TABLE XXI.

*Differences between Mean Radial Advance in Pairs of Samples of Bramley's Apples of Different Age. Strain MK.*

Sample.	A.	B.	C.	D.
B	0.0046			
C	0.0116	0.0070		
D	0.0318	0.0272	0.0202	
E	0.0587	0.0541	0.0471	0.0269

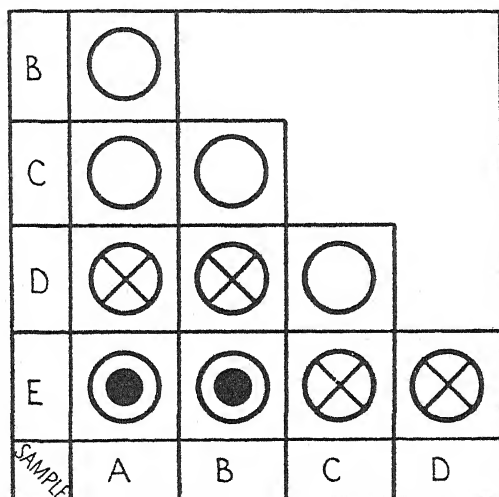


FIG. 12. Diagram showing degree of significance with age between pairs of samples of Bramley's Seedling apple, inoculated with MK, 1929. S.E. = 0.0083. For meaning of signs see p. 202.

It is seen from Fig. 12 that with MK, D and E are again the only consecutive samples which differ significantly. Significance of difference between other samples appears when differences in age are greater.

When the results of the three experiments are compared it is seen that with each strain there is significant difference in mean values of radial advance for the consecutive samples D and E, although the difference in their age is only seven days. Samples earlier and later than these, however, differ significantly only when the difference in age is three to four weeks. It is not unlikely that the age represented by samples D and E is the critical period, when the resistance of the Bramley's apple is rapidly falling.

## VI. DISCUSSION.

In 1922 Stevens (17) inoculated severed living shoots of certain cereals with seventeen saltants of *Helminthosporium*, and found them to vary in attacking power. In the following year Burkholder (3), in a paper on *Colletotrichum lindemuthianum* (Sacc et Magni) B. et C., states that the 'gamma' strain is more virulent than the 'beta' strain. The origin of the 'gamma' strain from the 'beta' strain was, however, assumed, but not established from experimental evidence. In 1925 Dickson (8) obtained a saltant of *C. atramentarium*, a fungus causing 'Black Rot' of Potatoes. It proved to be a weak parasite, but its attacking power was not compared with that of the parent strain. In 1926, Christensen and Stakman (6) recorded that the saltants which they obtained during the course of their work on *Ustilago zeae* differ greatly in pathogenicity and are less virulent than the parent. Leonian (16) in the same year stated that some saltants of *Phytophthora omnivora* are more virulent than others, but again no comparison with the parent strain was made.

Saltation resulting in the origin of strains showing increased attacking power has been recorded by Christensen (4) in 1925, who found that two mutants of *H. sativum* are decidedly more virulent than the parent with respect to both barley and wheat, especially the mutant M-40. When the attacking power of the parent was again compared with M-40 three years later the mutant proved to be less virulent than the parent.

In some of the cases mentioned above, the conclusions reached by the authors are based on very slender experimental evidence. In others the experimental results cannot be tested because the data provided are either insufficient, or of a purely qualitative nature unsuitable for statistical analysis, and hence the significance of observed differences cannot be ascertained. This is doubtless due to the difficulty in finding an accurate quantitative measure of attacking power, especially when the host material consists of either the entire plant or leaves. In the case of the apple fruit, this difficulty was overcome by Gregory and Horne, who, taking advantage of the fact that the apple is approximately spherical in shape, converted raw data of weight of rotted tissue into data of radial advance (expressed in terms of radius of apple). When the radius of the apple is known, the rate of invasion of a given fungus can be calculated. Owing to the further discovery that the values of radial advance tend to be normally distributed, it is possible to make valid comparisons of attacking power of strains by the usual method of statistical analysis.

As far as the writer is aware the statistical method was first applied to the study of the pathogenicity of strains by Horne and Gregory (15). Working with various saltants of *Fusarium fructigenum* they found that the saltants were either as virulent as, or less virulent than, the parent

strain. These results were confirmed and amplified by Harvey (11) in 1929.

Statistical methods have also been employed by Bonde (2) to compare the relative pathogenicity of the strains of *Alternaria solani*. Pathogenicity was estimated by measuring the lesion produced by the strains inoculated on potato leaves. The data were analysed by Love's modification of Student's Method, and showed that a saltant of *A. solani* (C-5) was significantly more virulent than the parent strain (C-2). The relative activity of other parent and saltant strains is difficult to ascertain from the author's account. Saltants seem to have been less virulent than the parents. The results obtained with potato tubers as host material were not treated statistically, but it appears from such information as is given that the saltant I-3, originating from a moderately virulent strain N-2, was weakly parasitic.

Statistical analysis of the data obtained in the present investigation shows that the saltants of *Cytosporina ludibunda* described in the preceding paper of this series differ greatly in attacking power. When the results obtained over three consecutive seasons are considered together, the saltants may be conveniently grouped as follows: Group I. Most active. CA<sub>1</sub>, CA<sub>2</sub>, and CA<sub>3</sub>; Group II. Moderately active. MK, CA<sub>4</sub>, CC, and C; Group III. Weak. CC<sub>2</sub>. This grouping should not be regarded as rigid since in certain circumstances saltants placed in Group II may be found to occupy places in Group I or III.

The saltants included in Group I (CA<sub>1</sub>, CA<sub>2</sub>, and CA<sub>3</sub>) are more virulent than the original strain (C) from which they were derived. In addition to these strains which proved more virulent than C irrespective of variety of apple and experimental conditions, certain other strains proved significantly more virulent than C in particular circumstances, e.g., CA<sub>4</sub> in late Bramley's, 1927; and CA<sub>4</sub> and MK in Worcester, 1929. In such cases the strains concerned would fall in Group I. It is interesting to note that the saltants in Group I (CA<sub>1</sub>, CA<sub>2</sub>, and CA<sub>3</sub>) arose along one particular line of descent, viz., C-CA-CA<sub>1</sub>, &c.

When the seasonal results obtained with a single variety of apple (Bramley's Seedling) are compared, it is seen that the order of attacking power based on estimates of the radial advance in apples which are regarded as of comparable age is not convincingly affected by season. The order in 1928 is actually the same as that in 1929. Certain differences in the order were observed, but when rigid tests were applied to the data it was found that no significance could be attached to the changes in relative position of strains. In Bramley's, CA<sub>1</sub>, CA<sub>2</sub>, and CA<sub>3</sub> were decidedly the most active strains, and CC<sub>2</sub> invariably proved very weak. The strains showing medium virulence differed in actual values of radial advance, but the observed differences in most cases were insignificant.

The results show that the order of attacking power is influenced by age of apple. Thus in late Bramley's, 1927, a real difference in the order was established by a significant increase in attacking power, shown by CA<sub>4</sub>, hence CA<sub>4</sub>, which occupies a place in Group II in early Bramley's, falls into Group I in late Bramley's. A similar example of a tendency to a change in the order of attacking power with age of apple was recorded by Horne (13) for *Fusarium fructigenum*, strains D and A, in Cox's Orange Pippin. In early Cox's, *Fusarium D* was found to be much more active than *Fusarium A*, but with the increasing age of fruit the difference in activity diminished. Horne has found subsequently that the relative attacking power shown by these strains tends to be reversed with the increasing age of Worcester Pearmain apples.

The change in the observed order of strains with the increasing age of fruit is due to a differential effect of age on rate of invasion of the strains concerned. Thus with strain CA<sub>4</sub>, mentioned above, the rate of invasion recorded for early Bramley's is 0.005 cm. per day and that in late Bramley's is 0.0439 cm. per day, whereas the corresponding rates recorded for CC are 0.0109 and 0.0388 cm. per day. With the former strain the rate has increased about eight times, and with the latter less than four times.

The general effect on rate of invasion of the increasing age of fruit (Bramley's Seedling) is shown by an analysis of data from consecutive weekly samples of inoculated apples. With every strain used the rate of invasion increases significantly with increasing age, confirming previous statements to this effect by Horne (12, 13). Thus the rate of advance of CA<sub>2</sub> increased nearly six times from 0.0601 (first sample) to 0.3410 cm. per day (last sample). Again, with CA<sub>4</sub> and MK, the rates observed for the last sample are fourteen times and seven times respectively those recorded for the first sample. The use of older Bramley's apples also seems to bring out differences between strains of *Cytosporina ludibunda* (e.g. CA<sub>4</sub> and CC, 1927), which cannot be detected when younger fruit is used.

Horne and Gregory (15), using Bramley's Seedling and Cox's Orange Pippin apples, found that the attacking power of strains is profoundly influenced by variety of apple. In this paper the effects of the Bramley's Seedling and Worcester Pearmain varieties on the strains of *C. ludibunda* have been compared. The results confirm those obtained by Horne and Gregory. It is found that the actual values of radial advance are different in the two varieties, especially in the case of CA<sub>4</sub> and CC<sub>2</sub>, where the differences are significant. The order of the strains is also substantially altered. The most important change is shown by CA<sub>4</sub>, which supersedes in pathogenicity CA<sub>2</sub>, consistently the most active strain in Bramley's, and falls in the same group with it.

Analysis of the combined data obtained in 1928 and 1929 show that although all the six strains had greater rate of attack in the latter year,

only with the two  $CA_4$  and  $CC_2$  were the differences significant. Since the cultures of  $CA_4$  and  $CC_2$  showed no sign of change in the nature of their growth, it is suggested that the results of analysis indicate a differential effect of season on the attacking power of strains.

It is clear from the preceding pages that the attack of apples by the strains is affected by age and variety of apple and perhaps to a certain extent by season. These factors are known to affect the chemical constitution of the apple (1) and the evidence suggests that the observed variations in rate of invasion are due to such changes in composition of fruit rather than to modification of the fungal strains.

## VII. SUMMARY.

The attacking power of certain saltants of *Cytosporina ludibunda* described in the second paper of this series, has been tested and compared with that shown by the parent strain by inoculating apples and making estimates of the rate of invasion of the tissues. The experimental work has extended over three years (1927-9). The significance of the observed differences has been tested by the usual methods of statistical analysis.

Certain saltants ( $CA_1$ ,  $CA_2$ ,  $CA_3$ ) proved to be more active than the parent, C, under the experimental conditions employed; others ( $CC$ ,  $CA_4$ , MK) usually showed a degree of activity similar to that of the parent, but under certain experimental conditions (apple variety)  $CA_4$  and MK were more active than C. Strain  $CC_2$  proved to be, on the whole, the weakest strain tested. The rate of invasion varied greatly, the most active strain being from 20 to 50 times more active than  $CC_2$ .

Both variety of apple and age of fruit have a differential effect on attacking power of strains. A differential effect of season on attacking power of strains is suggested by the evidence, but is not regarded as conclusively established.

It is clear that differences or changes in resistance of apples to invasion (probably conditions of chemical composition) rather than modification of the fungus, are responsible for the observed variation in rate of radial advance with variety and age of apple.

In conclusion, I wish to express my indebtedness to Dr. A. S. Horne for his constant help and for placing at my disposal some of the data incorporated in this paper. I also desire to thank Dr. F. G. Gregory for advice given in connexion with certain statistical analyses. Finally, my thanks are due to Professor V. H. Blackman for his helpful criticism and for providing the necessary facilities for carrying out the investigation.

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# Cytological Studies in Cotton.

## I. The Mitosis and the Meiosis in Diploid and Triploid Asiatic Cotton.

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With Plates VIII-XI and one Figure in the Text.

### CONTENTS.

	PAGE
I. INTRODUCTION AND HISTORY . . . . .	227
II. MATERIAL . . . . .	229
III. TECHNIQUE . . . . .	230
IV. MITOSIS IN THE DIPLOID AND THE TRIPLOID . . . . .	233
V. MEIOSIS IN THE DIPLOID . . . . .	234
VI. MEIOSIS IN THE TRIPLOID . . . . .	236
VII. GENERAL DISCUSSION . . . . .	239
VIII. SUMMARY . . . . .	246
IX. ACKNOWLEDGEMENTS . . . . .	247
LITERATURE CITED . . . . .	248

### I. INTRODUCTION AND HISTORY.

ONLY three cytological papers dealing with cotton have been published up to 1924.

Cannon (11) studied the spermatogenesis of a hybrid cotton (*Gossypium barbadense* L.  $\times$  *G. hirsutum* L.). Balls (1 and 2) studied *G. barbadense* L. These papers have only been available to the writer in abstract form, but from the review by Beal (5) they apparently contain little of interest so far as the present paper is concerned.

Several cytological papers about cotton have appeared since 1924 dealing both with (1) chromosome numbers, (2) species crosses, (3) mitosis, and (4) meiosis.

1. The chromosome numbers have been counted in some species, and it has been shown that cultivated cottons fall cytologically into two groups. (a) New World cottons with  $n = 26$ , and (b) Asiatic cottons with  $n = 13$ . (Denham (20), Nikolajeva (see (46)), Banerji (4), Longley (see (28)), Youngman (44), and Nakatomi (35)). Cannon found  $n = 28$  in an Egyptian-Upland cross, but in the light of subsequent work this number must now be considered inaccurate. Similarly the number  $n = 20$  recorded by Balls (3) for Egyptian cotton must also be due to faulty technique. Youngman (43) found from 8 to 13 chromosome bodies in the homotypic metaphase of *G. barbadense* L., which Denham, Nikolajeva, Beal, and the writer have found to be  $n = 26$ . Later the same author (44) found  $2n = 52$  for a variety of *G. barbadense* L. When Vukovic and Glisic (40) found  $2n = c. 52-56$  in *G. herbaceum* L. (an Old World cotton with  $n = 13$ ) it may be explained by the fact that this species has been confused with *G. hirsutum* L., an error which has frequently been made by taxonomists. Thus the writer has received several samples of seeds under the name of *G. herbaceum* from different botanical gardens in Europe, none of which have been found to correspond with *G. herbaceum* but most with *G. hirsutum*.

Of wild *Gossypium* species *G. Stocksii* M. Mast. from Arabia and North West India has  $n = 13$  (45). *G. Davidsonii* Kellogg from Lower California and Mexico and *G. lanceoforme* Miers (= *Thurberia thespesioides* A. Gray) from Arizona have both  $n = 13$  (Longley, see (22) and (23)). These numbers have been confirmed by the writer, and in addition the chromosome numbers of the following species have been determined. (1) *G. sturtii* F. v. M. from Central Australia  $n = 13$ , (2) *G. Klotzschianum* Andss. from the Galapagos Islands  $2n = 26$ , (3) *G. Harknessii* Brandg. from California and Mexico  $2n = 26$ , and (4) *G. tomentosum* Nutt. from the Hawaiian Islands  $2n = 52$ .

From the facts that *Erioxylum aridum* Rose and Standley from Lower Mexico will graft on New World cotton (S. C. Harland, personal communication), that the chromosome number is identical with other wild New World cottons, being  $2n = 26$ , and that hybrids have been obtained between *Erioxylum* and Asiatic cottons (*G. arboreum* and *G. herbaceum*) and New World cotton (*G. barbadense*), it appears that this species will have to be merged in *Gossypium*.

On the other hand, *G. Kirkii* M. Mast. from East Africa will neither graft nor hybridize with any known species of *Gossypium*, and these facts, in conjunction with certain marked taxonomic characters, led Harland (23) to consider that this should be excluded from the genus. This view is strengthened by the writer's determination of the chromosome number  $n = 12$  and  $2n = 24$ .

2. Species Crosses: Denham (20) figures 13 chromosomes from the meiosis of two different Asiatic hybrids, *G. arboreum*  $\times$  *neglectum* and *G.*



*cernuum* × *rudicum*. There is no description of their meiosis, so irregularities have most likely not been observed. All the species mentioned are, however, types of *G. arboreum* L.

A short report on the heterotypic and homotypic division in a hybrid between New and Old World cottons has been given by Nakatomi (l.c.). He finds generally 13 bivalents + 13 univalent chromosomes in the heterotypic metaphase.

3. The somatic mitosis has been described by Vukovic and Glisic (l.c.) in a 52 chromosome cotton. Denham (19) described the corresponding premeiotic division in *G. barbadense* L. Both authors find that the nucleolus secretes chromatin by budding, but whereas Denham found that the chromatin forms 'a continuous spireme', Vukovic and Glisic found that 'les chromosomes se forment chacun separement'. Youngman (44) has studied the mitosis in *Hibisceae*, and described it in detail in *Thespesia populnea* L., which is assumed to be closely related to *Gossypium*. He found 'a more or less continuous spireme', and in contrast to the above-mentioned authors he concludes that the nucleoli are being 'secreted by the chromosome bodies'.

4. Meiosis in *G. barbadense* L. (Sea Island) has been studied in detail by Denham (19) and Beal (l.c.). Although their drawings exhibit considerable similarity their interpretation of them is quite different.

Denham states: 'the reduction division takes place along normal telosynaptic lines', while Beal states that the figures between spireme and diakinesis 'make a telosynaptic interpretation . . . impossible'.

The great divergences between the above-mentioned workers upon a normal division process is undoubtedly due to difficulties inherent in the material. Thus Denham (19) writes 'the cotton plant with its large number of minute chromosomes and its complex cytoplasmic organization is by no means a suitable subject either for the establishment of new cytological theories or for criticism of existing hypotheses. The structures in question are barely within the limits of effective visibility and the possibility of subjective error is large.'

## II. MATERIAL.

The material used consisted of a triploid Asiatic cotton, which appeared as a sterile rogue in the experiments of Mr. J. B. Hutchinson.

Mr. Hutchinson has kindly given me the following information upon the genetic constitution of the triploid which arose in one of his cultures:

'Three strains of Asiatic cotton entered into the parentage of the Asiatic triploid.

(1) *G. herbaceum* L., a strain received from Turkestan under the number N289. Carries genes for yellow pigment in the corolla (Y) and

red anthocyanin in the calyx ( $R^e$ ). The plant entering into the ancestry of the triploid was grown under the reference number H3.

(2) *G. arboreum* L. var. *rubicunda* (Watt). An Indian variety. Carries genes for absence of yellow pigment from the corolla ( $y$ ), and presence of red anthocyanin pigment in corolla, calyx, and leaves ( $R$ ). The plant entering into the ancestry of the triploid was grown under the reference number N8.

(3) A multiple recessive strain extracted from a cross between *G. arboreum* L. var. *rosea* (Watt) and a closely related type of *G. arboreum* L. var. *Nanking* (Meyen). Carries genes for absence of yellow pigment from the corolla ( $y$ ) and total absence of anthocyanin from the plant ( $r^g$ ). The plant entering into the ancestry of the triploid was an  $F_4$  plant grown under the reference number N6-34. It has since bred true for all major characters.

A cross was made between H3 and N8, and an  $F_1$  plant, H3  $\times$  N8-2, was pollinated by N6-34. The triploid appeared as an exceptionally vigorous plant in the resulting family. The  $F_1$  H3  $\times$  N8-2 was a "red with yellow" flowered plant of the constitution  $RR^eYy$ , and the back-cross family gave four types of segregates in the following proportions:

	Red.	Red calyx.	Total.
Yellow	9	11	20
White	11	8	19
Total	20	19	39

The triploid was phenotypically "red calyx, white flower"'.

As the triploid showed none of the dominant factors from H3  $\times$  N8-2 but the two recessive genes  $R^e$  and  $y$ , of which  $R^e$  is dominant over  $r^g$  from N6-34, and they both have  $y$  in common, the triploid must undoubtedly have got 26 chromosomes from N6-34 and 13 chromosomes from H3  $\times$  N8-2.

Cytological examination confirms this explanation, in so far as it shows that there is normally full conjugation between the chromosomes in the hybrid H3  $\times$  N8-2. The appearance of the triploid is thus not a consequence of interspecific crossing, and it is probable that a 13 chromosome egg has either been doubly fertilized or more probably fertilized by an accidental diploid pollen grain. Throughout the work comparison has been made of the cytology of ordinary diploid Asiatic cotton (*G. arboreum* L.).

### III. TECHNIQUE.

The root-tips were fixed in Navashin's solution. Some slides were stained in gentian violet, others in Heidenhain's iron haematoxylin. Most of the reproduced figures are from slides stained in haematoxylin, which stains both chromatin and nucleolus. The shape of the nucleolus has been very useful in distinguishing the succession of the stages.

Unexpected difficulties were, however, encountered in obtaining fixed material illustrative of meiosis, especially in the triploid. Several authors have referred to difficulties in obtaining good fixation of a normal diploid cotton.

The writer agrees with Denham that meiosis in cotton is not an especially suitable subject for cytological study, but considers that he has somewhat over-stated the difficulties. These difficulties have been in large part overcome by certain modifications in technique and material. Thus, it was necessary to employ a better fixative and to use gentian violet as a stain instead of haematoxylin. This stain provides greater transparency together with sharp contrast between cytoplasm and chromatin. The heavily stained perinuclear zone in meiosis, which has been observed by previous workers, and which must have obscured the nuclear material, is not found with the modified technique. In this connexion Denham's (19) photographs Pl. XIV, Figs. 18 and 19, may be compared with the better fixed nucleus as Pl. XIV, Figs. 16 and 21. In badly fixed pollen mother-cells of difficult species of *Saxifraga* or hybrids thereof the writer has observed corresponding perinuclear zones which disappear by using better fixations. Further, Asiatic species (*G. arboreum* L. and *G. herbaceum* L.) are better adapted for study than the New World types, since they possess less chromatin material and only half the number of chromosomes. Lastly, a triploid Asiatic was available, and a cytological comparison of this type with the diploid gave critical evidence in the question of teloparasynapsis.

Preliminary experiments on the effects of different fixatives were therefore considered necessary, and between forty and fifty different fixatives or combinations of fixatives have been tried. The best results so far have been obtained by a prefixation in Winge's Picro-Carnoy (41), followed by a fixation in an osmic acid modification of Allen's Bouin, devised by the writer.

The standard method is set forth in some detail since the difficulties of cytological work in the Tropics require modification of the normal procedure adopted in the Temperate Zone.

1. Material is best collected between 8 and 11 a.m. in full sunlight. In collections made on cloudy days the fixation is inferior, and there seems to be a tendency for the chromosomes to clump and the cytoplasm to shrink.

2. After removal of the calyx and the top of the corolla the buds undergo a preliminary fixation in Winge's Picro-Carnoy solution:

8 per cent. mercuric bichloride in absolute alcohol . . . . .	— 1 part
5 per cent. picric acid in absolute alcohol . . . . .	— 2 parts
Chloroform . . . . .	— 3 parts

A mixture of 100 c.c. 7 per cent. urea in absolute alcohol, and 33 c.c. glacial acetic acid . . . -4 parts.

The temperature of the fixative should be 37° C., and fixation should last 5-15 minutes.

3. The buds are then transferred to another fixative, the method and preparation being as follows:

A. Saturated solution of picric acid in water . . .	75 c.c.
Glacial acetic acid . . . . .	5 c.c.
Urea . . . . .	2 gm.

Heat the above mixture to about 37° C., and add:

Chromic acid . . . . .	1.5 gm.
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B. 2 per cent. osmic acid.

C. 40 per cent. formalin.

Mix:

A. -3 parts

B. -1 part

C. -1 part

The fixative must be made up immediately before using. The fixed material is kept for 20-24 hours in an incubator at 37° C.

4. Washing was done in 70 per cent. alcohol.

5. The fixed material was carried through absolute alcohol and through mixtures of either alcohol-xylol or alcohol-chloroform to paraffin. It was apparently immaterial whether xylol or chloroform was used.

6. The material was embedded in paraffin of M.P. 56°-60°, and as soon as the surface of the paraffin had hardened it was put into ice water to secure rapid hardening.

7. The cutting of the material was then just as easy as with paraffin of M.P. 52°-54° in a colder climate. The sections were cut 20  $\mu$  thick to secure some untouched nuclei.

8. The paraffin was removed, and the slide transferred to 70 per cent. alcohol.

9. The bleaching was done according to Mayer's method (a few gm. chlorate of potash + a few c.c. nitric acid + c. 150 c.c. 70 per cent. alcohol). This method was found harmless and much more effective than that involving peroxide of hydrogen, which moreover has the disadvantage of not keeping well in the tropics.

10. The slides were then washed carefully in running water, and left overnight in a 1 per cent. aqueous solution of chromic acid. The treatment with chromic acid has a favourable effect on the staining process, and if the material is fixed in a fixative without chromic acid its use before staining is

essential, since otherwise a gentian-violet stain will stain both chromatin and cytoplasm to the same extent.

11. After five minutes washing in running water the slides were stained in gentian violet.

12. The slides were examined in xylol, and permanent preparations were mounted in Canada balsam.

The figures were drawn with the aid of a camera lucida employing a Zeiss oil immersion 1.3 mm. ( $\times 120$ ) and ocular ( $\times 20$ ). They are here reproduced at a magnification of about 3,200 times. The photographs were taken with Mr. Osterstock's cine-camera.

The terminology of the *Gossypium* species is according to Harland (23).

The cytological terms for the nuclear stages are mainly from Belling (8). The term zygotene is used as an equivalent to synizesis, and the term diplotene is used for all stages between pachytene and metaphase, as suggested by Catcheside (12). The remainder of the cytological terminology is according to Darlington (17).

#### IV. MITOSIS IN THE DIPLOID AND THE TRIPLOID.

The following description is based on the diploid. The triploid is only mentioned when differences other than quantitative ones are involved.

In good metaphase plates it is occasionally possible to distinguish different types of chromosomes.

Pl. VIII, Fig. 1 shows such a metaphase with 26 chromosomes of which 4 have a satellite. In the triploid it is impossible to get all the 39 chromosomes in a sufficiently favourable position to study their shapes. In the triploid metaphase shown in Pl. VIII, Fig. 2, it is thus not possible to demonstrate 16 chromosomes with satellites. Pl. VIII, Fig. 3 shows an anaphase from the diploid where the chromosomes are passing to the two poles. In Pl. VIII, Fig. 4, the chromosomes are seen from the pole at a little later stage, where they have started to fuse together, but otherwise have retained their normal shape, no splitting being observed. In Pl. VIII, Fig. 5, the fusion is so complete that it is no longer possible to demonstrate single chromosomes. The following opening up of the chromatin material is shown in Pl. VIII, Fig. 6, which is reminiscent of a spider's web with two heavily stained nucleoli of varying shape as denser aggregations in the middle. Pl. VIII, Fig. 7 gives a later stage in which there is formed a nuclear membrane round the new nucleus. The chromatin has been collected more together in spots and the connexions between the spots and the nucleoli have lost some of their staining power. In a little later stage, Pl. VIII, Fig. 8, about 26 chromatin bodies are spread out in the plasma without any visible connexions at the same time as the nucleoli have

started to fuse. There is only a very short step to the resting stage which is observed between the premeiotic division and the meiosis Pl. VIII, Fig. 15. The corresponding stage is seldom observed in root-tips as the divisions generally follow rapidly after one another. A single heavy staining nucleolus is constantly present from the stage between telo- and prophase to the stage immediately before metaphase. The prochromosomes which are observed in a resting nucleus as in Pl. VIII, Fig. 9, become much bigger during the prophase. The connexion between them becomes clearer and they show a tendency to arrange themselves into pairs which often closely resemble the meiotic diakinesis. Pl. VIII, Fig. 10 shows an early prophase in the diploid, and Pl. VIII, Fig. 11 shows a little later stage in the triploid where the arrangement into groups consisting of one, two or three prochromosomes may be seen.

This pairing of the prochromosomes is of rather short duration and is followed by a stretching and separation until all 26 chromosomes are separately arranged in the nuclear periphery as in Pl. VIII, Fig. 12. The pairing in the prophase is to be traced in the metaphase by a more or less striking tendency of chromosomes of the same shape and size to lie together, as in Pl. VIII, Fig. 1, in which the two sets with satellites and several other sets of two may be seen lying together. As the chromosomes pass over in metaphase the nucleolus disappears and only occasionally small remains may be found during the metaphase, as seen in the middle of Pl. VIII, Fig. 1.

The premeiotic division is identical with the somatic mitosis, so far as it has been possible to follow it. Pl. VIII, Figs. 13 and 14 are from the premeiotic division respectively in the triploid and the diploid and correspond to Pl. VIII, Fig. 12, from the somatic mitosis.

## V. MEIOSIS IN THE DIPLOID.

Meiosis was studied both in the type N6-34 (*G. arboreum* L.) and the hybrid (H3 × N8) (*G. herbaceum* × *G. arboreum*). The following description applies to both types, unless a statement is made to the contrary.

The resting nucleus as it appears after the premeiotic division shows a large heavily stained nucleolus and only very little chromatin (Pl. VIII, Fig. 15). The amount of chromatin increases with the volume of the nucleus and becomes arranged into a network which covers the surface of the nucleus (Pl. VIII, Fig. 16). As the nucleus becomes larger the network becomes more distinct and takes in the form of a single thread with a structure resembling that of a necklace (Pl. VIII, Fig. 17). It is impossible to say whether it is a continuous thread or not as there are too many anastomoses. In the subsequent contraction the thread generally arranges itself round the nucleolus (Pl. IX, Fig. 18). Both in Pl. VIII,

Fig. 17, and in Pl. IX, Fig. 18, parallel arranged threads may be found, but it is by no means a characteristic phenomenon for these stages. During this contraction the threads must be supposed to pair to form a double thread. Pl. IX, Fig. 19 shows zygotene formed of a much heavier thread than that shown in Pl. VIII, Fig. 17, and in Pl. IX, Fig. 18, and its double nature can be seen at *x*. The double nature of this strand is much easier to see in early pachytene stage, as in Pl. IX, Fig. 20.

The strands become more spread out until they lie near to the surface of the whole of the nucleus.

At this stage (Pl. IX, Fig. 21) it is possible definitely to demonstrate the free ends of the strands. It is also possible to see where two pieces of the strand have conjugated end to end (cf. Pl. IX, Fig. 21, with Pl. XI, Figs. 48-52), and as the plasmasomes must be the ends of segments, as in tulips (36), it is theoretically possible to count the whole number of segments in the pachytene strands.

Unfortunately the writer has not succeeded in finding a nucleus in which the number could be clearly demonstrated. But in an untouched nucleus, as that in Pl. IX, Fig. 21, it is possible to follow the strand so well that the number of segments can be approximately determined. It is here clear that the number must be very close to 13 and cannot be 26. *This indicates that each piece of the strand corresponds to a pair of chromosomes, and a single thread must correspond to a whole chromosome.*

The shortening and thickening of the chromosomes from pachytene to metaphase comes on rather suddenly. Many of the nuclei show therefore several stages. Pl. IX, Fig. 22 shows a part of a nucleus in which a shortening has begun in most of the threads. In Pl. IX, Fig. 23, is shown a little later state in which the 13 chromosome pairs are separated. Pl. IX, Figs. 24 and 25 show two sections of a nucleus in which the chromosomes represent different stages in the condensation. Simultaneously with the condensation of the chromosome loops, the separation between the partners increases and in the middle diplotene it is sometimes possible to distinguish the four chromioides in a pair of chromosomes (Pl. IX, Fig. 25). The number of chiasmata in a pair of chromosomes varied from 1 to 3. In the late diaphase (Pl. IX, Figs. 26 and 27) and metaphase only one or two chiasmata may be distinguished, of which two terminal chiasmata is the more frequent condition. The chromosomes appear in a polar view focused at their middle as two circles which may be quite separated (Pl. IX, Fig. 28).

In this chromosome plate all the partners must have formed at least two chiasmata, which seems to be a common phenomenon in a normal diploid Asiatic cotton. In the interspecific hybrid the number of chiasmata is less (Pl. IX, Fig. 29), so that both rods and rings are common. A single pair is characterized by a conjugation so loose that most likely the

chiasma is occasionally not formed, which will result in univalent chromosomes, as the second division indicates. Pl. XI, Figs. 53-8 are from metaphases of the first division in the interspecific hybrid. Pl. XI, Figs. 53-5 are side-views in which the ring-shaped chromosome pairs may be seen. Pl. XI, Figs. 56-8 are polar views from which the 13 pairs may be counted. As the chromosomes are drawn out in anaphase (Pl. IX, Fig. 30) their shapes soon change so much that their individuality in polar view no longer can be traced (Pl. IX, Fig. 31). The subsequent transition is difficult to follow owing to the rapidity with which it takes place, together with the small size of the structures in question. The chromosomes break up into granules which arrange themselves in threads (Pl. IX, Fig. 32) out of which double chromosomes are formed (Pl. IX, Fig. 33, and Pl. X, Fig. 34). The double nature of the chromosomes is specially characteristic just before metaphase of the second division, but even in a polar view of the metaphase it can easily be traced (Pl. X, Fig. 35). The separation of the chromosomes takes place regularly and four nuclei are formed.

To get some idea of the regularity with which the first meiotic division takes place, a number of metaphases from the second division were counted. In the type N6 no irregularities were observed (which corresponds very well with the observation), there being generally two chiasmata in each chromosome pair at metaphase and no evidence of univalents. The hybrid plant H3 × N8 showed as above mentioned fewer chiasmata at the first metaphase. In 46 pollen mother-cells 64 metaphases from the second division were counted; 60 of these had each 13 chromosomes (cf. Pl. XI, Figs. 59-62), 2 had 12 and 2 had 14 chromosomes. The photographs (Pl. XI, Fig. 63-6) are from a pollen mother-cell representing the metaphase of the second division in which 12 chromosomes can be counted in one and 14 chromosomes in the other plate.

## VI. MEIOSIS IN THE TRIPLOID.

No differences in quality between the diploid and the triploid have been observed in the stages leading to zygotene, though quantitative differences exist. In the loosening out stage leading from zygotene to pachytene, strands of different thickness may be observed, but it is only in the few cases where the strands open up that it is possible to distinguish the number of elements comprising each strand. Special attention has been given to the determination of the number of threads in the strand, which emerges when the zygotene knot starts to open. At this stage there is no possibility of confusing the conjugation with an eventual 'second contraction'. In Pl. X, Fig. 36, is shown such a nucleus in which the first strand emerging with a free end consists of three threads, though most of the other strands are impossible to analyse. A corresponding nucleus



is shown in Pl. XI, Figs. 69–85. Pl. XI, Figs. 69–71 give pictures of the nucleus where the nucleolus and a pachytene which has started to loosen up may be seen. Pl. XI, Figs. 72–85 are a cinematic series taken with very small alterations of the focus for each photograph. They show the pachytene strand which has come farthest out from the zygotene knot, and it is here possible to recognize that one of the ends of the pachytene strand is free and that it is made up of three threads. Pl. X, Fig. 37, shows a part of the nucleus in early pachytene where two strands can be distinguished consisting each of three threads, while others undoubtedly consist only of two. Pl. XI, Figs. 62–8 show another triple pachytene strand. In Pl. X, Fig. 38, are shown two strands from pachytene each consisting of two threads. The threads from one pachytene strand have conjugated with the threads from the other, one of them for so long a stretch that there must be possibilities of the formation of tetravalent chromosome configurations. The pachytene stage is too complicated in the triploid to render similar analysis as done in the diploid.

The diplotene is much easier to analyse as shown in Pl. X, Fig. 39. There are here 2 univalents, 4 bivalents, 3 trivalents, 1 tetravalent, 2 pentavalents, and a hexavalent.

In Pl. XI, Figs. 86–98, is shown the diplotene of a tetravalent, where each chromosome pair is forming three chiasmata. Pl. XI, Figs. 90–3 show a pentavalent arrangement from the same stage.

The metaphase is still easier to analyse when it is seen from side-view. In Pl. X, Fig. 40, all the chromosome configurations are drawn separately to show the chromosome conjugations. There are 2 univalents, 4 bivalents, 7 trivalents, and 2 tetravalents. Some of the higher chromosome configurations are shown separately. Pl. X, Fig. 41, shows four types of trivalents not represented in Pl. X, Fig. 40. Pl. X, Fig. 42, shows five tetravalents, Pl. X, Fig. 43, two pentavalents, Pl. X, Fig. 44, three hexavalents, and Pl. X, Fig. 45 shows a septavalent, which represents the highest chromosome configuration observed.<sup>2</sup> Furthermore, some of the photographs illustrate the chromosome conjugations. Pl. XI, Figs. 94–7 show 2 univalents, 1 bivalent, 2 trivalents, and a tetravalent in which the chromosomes are arranged in a zig-zag. Pl. XI, Figs. 98–101 show a pentavalent consisting of a zig-zag of 3 chromosomes attached to a normal-looking pair, at the end of which a univalent chromosome is situated. A trivalent is seen at the other end of the pentasome. Pl. XI, Figs. 102–4 show a tetravalent between two trivalents. In Pl. XI, Figs. 105–7, are seen 1 univalent, 4 trivalents, and a string probably consisting of 5–6 chromosomes.

To get some idea of the general chromosome conjugation, 20 pollen mother-cells were analysed and the results are given in Table I. It is seen that out of 20 pollen mother-cells only two showed a chromosome conjugation which could normally be expected (i.e. a range from 13 trivalents

to 13 bivalents + 13 univalents). Fourteen of these show higher chromosome configurations than trivalents.

*Table Showing Chromosome Conjugation in 20 Pollen Mother-cells in the Triploid.*

Pollen mother-cell.	Uni-valent.	Bi-valent.	Tri-valent.	Tetra-valent.	Penta-valent.	Hexa-valent.	Septa-valent.
1	2	4	7	2			
2	3	7	6	1			
3	1	7	5	1	1		
4	3	8	5		1		
5	6	12	3				
6	2	2	6	2			1
7	3	18					
8	2	2	11				
9	1	4	10				
10	3	15	2				
11		2	9	2			
12	4	3	8		1		
13	1	4	6	3			
14	1	1	12				
15	1	3	8	2			
16	2	4	3	1	2	1	
17	4	5	3	4			
18	5	5	2	2	2		
19	4	5	7	1			
20	2	3	7	1		1	
Total	50	114	120	22	7	2	1

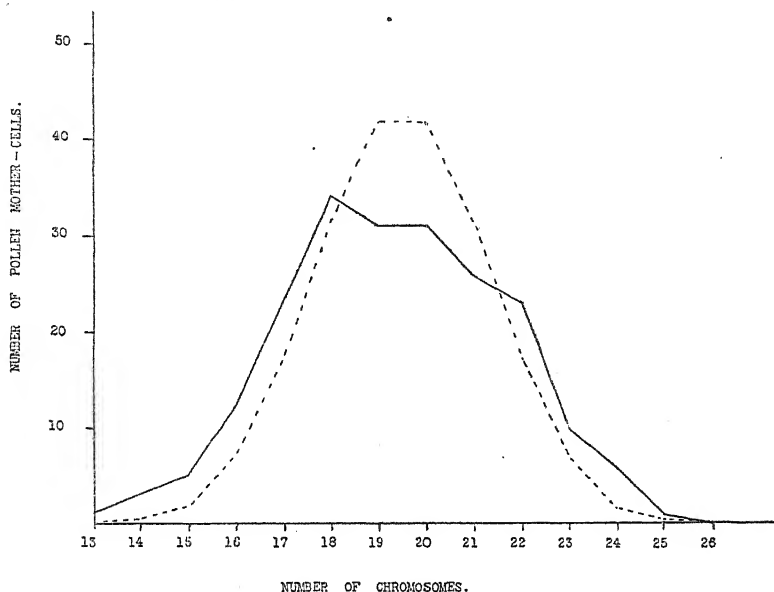
Subsequent stages resemble very much what already have been described in the diploid. The only observed difference is in the inter-stage. Here the diploid showed generally no nucleolus, but a single small nucleolus was occasionally present (Pl. IX, Fig. 33), while the triploid showed a large number of nucleoli of different sizes (Pl. X, Fig. 46).

No splitting of single chromosomes has been observed during the first division, and observations from the second division demonstrate that splitting must occur seldom, if at all. It was thus possible in 40 pollen mother-cells showing metaphases at the second division to count all the 39 chromosomes.

The second division seems quite regular, and in about 95 per cent. of the pollen mother-cells only 2 chromosome plates were observed, which shows a regular distribution of all the chromosomes to two poles at the first division (see Pl. XI, Figs. 108-113). Several hundred pollen mother-cells have been examined, but only in about 5 per cent. were 3 chromosome plates observed. Only one of these showed 3 chromosome plates of about the same size, while in the remaining cases all showed two big groups and a small group consisting of from 1 to 3 chromosomes. Pl. X,

Fig. 47, shows such a case with 21, 17, and 1 chromosome, most of which show the longitudinal splitting very clearly.

As exceptions may be mentioned that in two cases all the chromosomes from the second division were arranged in one plate. In one of



Number of chromosomes observed in 200 metaphases from the second division in the triploid (full drawn line) compared with the expected in a triploid in which the extra set was distributed at random (stippled line).

these cases the cytoplasm showed clearly that two poles had been formed at the first meiosis, but that all the chromosomes were arranged in a single plate just in the middle between the two poles.

Two hundred metaphases from the second division were counted to give an idea of the number of chromosomes in the pollen. Only pollen mother-cells which had two chromosome plates were used (full drawn line in the text-figure) to secure comparison with expectation in a triploid in which the extra set was distributed at random (stippled line in the text-figure).

As a result of the meiosis a tetrad is generally formed. Exceptions occur only where irregularities had occurred in the first division resulting in a hexad, or where two metaphases had fused in a second division to form a diad. The size of the pollen grain depends on the number of chromosomes which it contains.

## VII. GENERAL DISCUSSION.

All the structures which have been dealt with in the present study are much smaller than those in more favourable material. The length of the

somatic chromosomes is always less than  $3.5\mu$ , whereas the somatic chromosomes in *Fritillaria* have an average length of  $18\mu$  (16). The meiotic chromosomes in Asiatic cottons are about  $2\mu$  long, while, for instance, *Gasteria* has chromosomes up to  $10\mu$  long and  $3-4\mu$  thick (13). The diameter of the single thread in the pachynema strand is about  $0.1$  to  $0.2\mu$ . 'The limit of resolution of lines is theoretically given as  $0.21\mu$  for vision and  $0.15\mu$  for photography' (7). When working near the limit of microscopic resolution it is clearly useless to attempt to elucidate the finer details which have been observed in more favourable material. The discussion is therefore confined to a comparison between the conclusions of previous workers and the present observations. The results recently obtained from studies of more favourable material have been taken just as much into consideration as the results obtained by previous workers in cotton.

#### A. Somatic Mitosis.

A number of theories on the relationship between the nucleolus and the chromatin have been advanced (see 47). Two of these have been emphasized in the literature discussing the somatic mitosis in the *Hibisceae*. Denham (19) and Vukovic and Glisic (l. c.) state that the nucleolus forms the chromosomes, while Youngman (44) maintains that *Thespesia* supports the idea that the nucleolus is formed by the excretion of substance from the chromosomes. The observations here described support the view that there is some connexion between the nucleolus and the chromatin, at any rate during telophase. The fact that gentian violet stains chromatin but scarcely either nucleolus or linin with the fixation technique here used for root-tip material, argues against both the above theories. Haematoxylin, however, stains both nucleolus and chromatin, just as is the case with gentian violet when used according to the technique here described for the buds or applied to the root-tips when Johanssen's (26) staining method is applied. There must therefore be a slight chemical difference between chromatin and nucleolus which argues against a direct excretion either way of the two mentioned. The close relationship between chromatin and nucleolus seems a too fundamental question to justify any general conclusion here. It has not been sufficiently elucidated in other material and the observations in cotton are so far very limited. In contrast with Denham, the observations of Vukovic and Glisic and those here described agree in that a continuous spireme has not been found. When Youngman (44) describes a continuous spireme in *Thespesia*, it may be mentioned that his figures show no justification thereof. Further, the assumption in his summary of 'a more or less continuous spireme' seems logically an impossibility.

The tendency here described for the chromosomes to pair in early prophase has not been reported before in cotton. Corresponding observa-

tions have, however, been made in several other plants (39), and the phenomenon seems very common in Diptera. It is undoubtedly this affinity which results in the two homologous chromosomes often being seen close together in the somatic metaphase as in most figures from *Drosophila*.

It may be mentioned that the number of nucleoli in the root-tips is always both in the diploid and the triploid in each cell only one during resting and prophase stages. De Mol (34) has on the contrary found that the number of nucleoli increases in proportion to the number of chromosomes.

### B. Meiosis.

There is full agreement between the observations of Denham (19), Beal, (l. c.), and these here described about the stages leading to pachytene. The interpretations of the observations are, however, different. Denham regards the pachytene strand as formed by closely associated half-chromosomes, while Beal explains the pachytene as consisting of paired chromosomes. *The fact that the triploid shows pachytene strands composed of three threads together with the observation that it has been possible to count the number of pachytene strands approximately in the diploid, leave no doubt that the pachytene strands represent whole paired chromosomes.* The supposed 'second contraction' which Denham (19) describes must undoubtedly be due to a misinterpretation of the observed stages, as Beal already has pointed out. In contrast to the authors who describe the pachytene strand as forming a 'continuous spireme', the pachynema strands here described are terminated by (1) free ends (cf. Pl. IX, Fig. 21, and Pl. X, Fig. 36, and Pl. XI, Figs. 51-2 and 72-3), (2) plasmasomes (Pl. IX, Fig. 21) or (3) two pachytene strands paired end to end (Pl. IX, Fig. 21, and Pl. XX, Fig. 38). Free ends have been described from most species in which parasynapsis has been observed to take place, such as tulip (36) or hyacinth (15). Plasmasomes are described by Denham (19) in Sea Island cotton ( $n = 26$ ), where there is anastomosis of the strands, and they are to be seen in his figures of spireme. Newton (l. c.) has described plasmasomes at the end of free pachytene strands in tulips. The difference between the observations of a 'continuous spireme' in the New World cotton (19 and 5) and free ends, as in Asiatic cotton, may be due to (1) difficulties in observing free ends in the much longer thread or (2) to the fact that the pairing end to end of the pachytene strands is so much more frequent in the higher chromosome numbered species that free ends are very few, or alternatively do not occur at all. The basis for this end-to-end conjugation must most likely indicate a type of homology either (1) due to a kind of 'secondary pairing' or (2) a homology produced from segmental interchange.

The stages which the pachytene strands pass through to metaphase as they shorten and contract correspond to what has been described from

large chromosomed plants as hyacinths (15). Beal's description of the parallel stages in New World cotton differs materially from these. He describes and figures the chromosome formation as a direct transverse segmentation of a heterogeneous bivalent spireme where most of the shortening and contraction have taken place before the segmentation. These stages are undoubtedly the most delicate to fix, so it must be much more difficult to follow the process in the higher chromosomed species.

No more than three chiasmata have been observed in a pair of chromosomes in the stages from diplotene to metaphase. The terminalization of the chiasmata is not always complete, so that sub-terminal to interstitial chiasmata may be seen at metaphase in the diploid (Pl. IX, Fig. 29). Real interstitial chiasmata seem more frequent in the triploid (Pl. X, Figs. 40 and 42).

The pairing of the chromosomes at metaphase appears to be quite normal in the diploid *Gossypium arboreum*. Two terminal or sub-terminal chiasmata are formed in most cases, so that a pair of chromosomes appear as a ring. The diploid *herbaceum* — *arboreum* hybrid showed generally at metaphase a less number of chiasmata in each pair of chromosomes, so that pairs having the appearance of rods instead of rings were more frequent here than in the pure species. In most cases the conjugation was, however, strong enough to form thirteen pairs at metaphase. It may be mentioned that it is the writer's impression that the doubleness of the pachytene strands was much more easily observed in the hybrid than in the pure species, due to the fact that the threads were separated over longer distances. The occasional lack of pairing in the hybrid may either be explained by: (1) That the two Asiatic species have arisen in different ways (see later), or (2) the types have been geographically separated sufficiently long for alterations to have taken place in the chromosomes in such a way that only short pieces have remained homologous so that chiasmata are not always formed.

The literature describing the stages leading to metaphase of the first division in a triploid is very limited. The chromosome conjugations have, on the contrary, been the subject for much more numerous studies. The following types of chromosome conjugation have been described:

(1) Only uni- and bivalents are formed: *Drosera* (38) and *Solanum nigrum* (27).

(2) A mixture of uni-, bi-, and trivalents are formed.

This is the most common group of which a number of cases have been described, e.g. tomatoes (31 and 27) and *Zea* (33).

(3) Only trivalents are observed: *Datura* (9).

(4) Higher chromosome configurations than trisomes are formed or a larger number of bivalents plus trivalents are formed than the basic chromosome number of the group.

The cotton triploid belongs to the last-mentioned group. Yarnell (42), in a study of triploid *Fragaria* which, according to his description, belongs to this group, has given a list of similar cases. As most of these have not been available to the writer they will not be dealt with here. Some of the examples have, however, arisen in the wild state (e.g. *Rubus*), or are in other ways too little elucidated (e.g. *Oenothera*, compare (18) footnote, p. 143) to give much support to the theory of pairing between non-homologous chromosomes.

The described chromosome conjugation in the triploid is in striking contrast to normal pairing in the pure species and the weaker pairing in the interspecific hybrid in which occasionally univalents must occur. The conjugations in the triploid must then be due to the triploid offering possibilities which must also be present in the diploid, although they here normally do not become realized. Or to express it in genetical terms: The pairing in the diploid shows only a 'phenotypic conjugation', while the triploid exhibits more of the 'genotypic conjugation': i.e. the greatest possible amount of conjugation is realized when all homologous parts have chiasmata.

The reasons for omission of possible conjugations have so far been very little elucidated. But cases such as *Solanum nigrum*, where the diploid type forms normal bivalents while the haploid shows autosyndesis (27); diploid *Crepis capillaris*, arisen by doubling in a haploid, shows gradation from full pairing to all chromosomes univalent (25); and the hybrid *Pygaëra pigra*  $\times$  *P. curtula* which gave practically no pairing in the male but full pairing in the female (21); these are only a few cases of the many described which show that the observations give only the 'phenotypic conjugation' which may not indicate the 'genotypic conjugation'.

The chromosome conjugations of the triploid may be explained in two ways: (1) As the result of segmental interchange, or (2) due to thirteen not being the basic number of the group but a balanced polyploid number.

1. Segmental interchange between non-homologous chromosomes would most likely give ring formation in the diploid as, for instance, shown in *Pisum* (24), but only normal pairing is observed here, and the interspecific hybrid has still weaker chromosome pairing. The fact that a balanced polyploid (e.g. *Solanum nigrum*) normally only shows bivalents agrees, on the other hand, very well with these observations and supports thus the polyploid explanation. Most of the polysomes may quite well be explained as the result of segmental interchange, but the bigger number of bi- plus trivalents than thirteen makes an explanation based on polyploidy much more probable.

2. The possibility that cottons with  $n = 13$  are secondary polyploids has recently been advanced by Lawrence (30). On the basis of Denham's (19) figures he says: 'Association in the  $n = 13$  forms suggests that these,

like *Pyrus*, are secondary polyploids in which certain chromosome types have been duplicated', and later he continues, 'the complex results obtained in breeding experiments on such plants as Cotton (Harland, 1929-30, Kearney, 1930) are all indications of polyploidy'. Although the writer agrees with Lawrence that Asiatic cottons are polyploids, it is for quite different reasons. The writer has in vain looked for secondary pairing in Asiatic cotton. No metaphase figures have so far been found which are more convincing than Denham's figures. There is here a variation from all chromosome pairs connected ((20), Fig. 7, upper row) to all chromosome pairs without any connexions and about the same distance between each pair ((20), Figs. 10-11, lower row). No such figures as those of *Dahlia* (29) or *Pyrus* (18) have been observed. As to the genetical evidence for polyploidy, it may be mentioned (1) that the presence of modifying factors does not prove polyploidy, and (2) the results quoted from Harland are not from Asiatic but from New World cottons ( $n = 26$ ), which must be definitely polyploids. Hence Asiatic cottons here are regarded as polyploids. Furthermore the described end-to-end conjugation of pachytene strands and the occurrence of a few trisomes in a haploid New World cotton (unpublished data) confirms the view.<sup>1</sup>

The two species of Asiatic cottons may then, for instance, be explained as polyploids arisen after the crossing of two different but closely allied 7-chromosome species with the same 6-chromosome species. The results resemble those of *Viola tricolor* where Clausen (14) has shown that the set of 13 chromosomes consists of one set of A (7 chromosomes) and one set of B (6 chromosomes). *Viola* and cotton differ in that cotton shows autosyndesis in contrast to *Viola tricolor* where the two sets are dissimilar. Alternatively it may be suggested that the complement of 13 is composed of two similar sets of 6, and one chromosome appearing a third time. Too few chromosome numbers are, however, known from the *Malvaceae*<sup>2</sup> to justify any definite conclusion yet.

The distribution of univalent chromosomes to the two poles has been shown statistically to take place at random in haploid *Datura* (10). It has been shown in triploid *Datura* (9), triploid hyacinths (6), and triploid *Solanum* (31) that the distribution of the chromosomes is a whole set to each pole, and the third set distributed at random. The distribution in

<sup>1</sup> Duplication of chromosomes should result in some cases of duplication of genes. The following two cases have kindly been given me by Dr. S. C. Harland (New World cottons) and Mr. J. B. Hutchinson (Asiatic cottons). Duplicate genes for brown lint colour in Asiatic cottons, triplicate genes for chlorophyll deficiency, duplicate genes for red, and duplicate genes for brown lint colour in New World cottons.

<sup>2</sup> The writer has found the chromosome number 7 or a multiple thereof in a number of *Malvaceae* such as: *Abutilon*, 7, 21; *Lavatera*, 7, 21, 42; *Malva*, 21, 42; *Pavonia*, 56; *Sida*, 7, 14, 28; *Urena*, 14; *Hibiscus*, 28, 42; and a multiple of 6 in: *Malva*, 12; *Sida*, 12; *Hibiscus*, 18, 36, 42 and *Gossypium Kirkii*, 42.



the triploid cotton has been studied by counting 200 metaphases from the second division. In the text-figure the full drawn line illustrates the observations, while the stippled line indicates the theoretical expectation if cotton behaved in the same way as *Datura* and *Solanum*. The difference between the two curves is systematic and bigger than may be explained by chance. Autosyndesis in the set of 13 chromosomes must cause the discrepancy. One would expect to find the chromosomes more equally divided between the poles, but a deficiency is found near the 18 and 19 and an excess near both limits. The hypothesis of a lethality of pollen cannot be applied here as the observations are from the second division which exhibit no such signs. Chromosome plates with 18 or 19 chromosomes are not more difficult to count than those with higher numbers. The whole of the curve has, however, been pushed slightly downwards for the reason that chromosome plates of number near 13 are more likely to occur, and these can be counted with greater accuracy than those with higher numbers. The difference is, however, very small. The number of configurations containing more than one chromosome in each of the 20 pollen mother-cells (from the Table on p. 238) is as follows:

Number of chromosome configurations higher than univalents:

	11	12	13	14	15	16	17	18
Number of pollen mother-cells:	3	3	7	4	1		1	1

The difference between the observations and the expected distribution may then be explained as the result of the formation of numbers of chromosome configurations less than 13 in a pollen mother-cell together with an irregular distribution to the two poles of the chromosomes in the polyvalents.

### C. Cytogenetics

Most of the recorded triploids have been sterile, and the cotton triploid is no exception as it has also proved to be completely sterile when used as male or female parent. The formation of pollen grains with 39 chromosomes offers some hope of overcoming the sterility. Of several hundred pollen mother-cells showing the second division only two have been observed which might have formed well-balanced cytological types probably able to function. There seems also a chance, although a small one, of making a tetraploid Asiatic by crossing normal diploid Asiatic cottons with pollen from the triploid.

It may be mentioned that an Asiatic cotton with  $2n = 52$  must also be able to arise from somatic doubling of the chromosomes. The writer has thus observed an island of 52-chromosome tissue in the root of another type of *Gossypium arboreum* (Burma laciniated). It may be worth while looking for tetraploid Asiatic cotton plants in commercial fields. Such

plants will most likely be very vigorous and fairly sterile, and should therefore be easily noticed.

### VIII. SUMMARY.

1. A technique developed specially for cotton and adapted to a tropical climate has been described in detail.

2. The genetical constitution of a triploid Asiatic cotton appearing as a sterile rogue in culture was studied. It is compounded of 13 chromosomes from the female and 26 chromosomes from the male parent.

3. The somatic mitosis has been described. The following details are of main importance: (a) The somatic chromosomes are very small and uniform. In the diploid four chromosomes may be identified by having a small satellite. (b) Chromatin and nucleoli show connexions in telophase but their closer relationship has not been solved. (c) A tendency to a loosely paired arrangement of the chromosomes in somatic mitosis has been observed. A trace thereof is seen at metaphase where homologous chromosomes often are observed arranged together. (d) Only one nucleolus is observed in each nucleus during resting and prophase stages in root-tips of diploids and the triploid and from tetraploid tissue, contrary to de Mol's observations from tulips.

4. Meiosis has been described from diploid *Gossypium arboreum* and the interspecific hybrid *G. herbaceum* × *G. arboreum*.

The following main observations may be cited: (a) The pachytene strands are double and have undoubtedly arisen from pairing of leptotene threads. (b) The pachytene strands are terminated by (1) free ends, (2) plasmasomes, or (3) end-to-end conjugation of two strands. (c) The number of pachytene strands is close to 13, which demonstrates that each strand represents a pair of whole chromosomes. (d) The stages leading from pachytene to metaphase represent a shortening and condensing. Traces of longitudinal fission in the single chromosomes may be seen at diplotene. The number of chiasmata is never more than three in each pair. Terminalization is not complete, so that sub-terminal chiasmata are observed at metaphase. (e) *Gossypium arboreum* forms 13 pairs at metaphase, each having two terminal or sub-terminal chiasmata (rings). (f) The interspecific hybrid shows always at least one pair of chromosomes with no more than one chiasma (rod). (g) The second division is regular. The occurrence of chromosome plates with 12 and 14 chromosomes in the interspecific hybrid demonstrates that irregularities must have taken place during the first division.

5. The meiosis in the triploid is described in comparison with that of diploids. The following differences may be mentioned: (a) Pachytene strands composed of two and three threads have been described. Supposed univalent threads are noticed, and end-to-end conjugation of strands have

been described. (b) The first metaphase shows, as expected from the observations of pachytene, a mixture of uni-, bi-, and trivalents. Furthermore, polysomes up to septavalents are found, and a number of bivalents plus trivalents ascending to 17 and 18 has been observed. The conjugations demonstrate that autosyndesis between the chromosomes in a set of 13 must take place. (c) The second division is regular and shows that a distribution of all the chromosomes to two poles during the first division must have taken place in about 95 per cent. of cases. In the remaining 5 per cent. of cases three nuclei are formed, generally two big and a small one only containing 1 to 3 chromosomes. In two cases a fusion of the two chromosome plates during the second division has been observed. Pollen grains with 39 chromosomes are undoubtedly formed from such cases. (d) The chromosome numbers of 200 metaphases from the second division were counted. The results show that the distribution of the chromosomes to the two poles in the triploid Asiatic cotton is not the same as found in triploid *Datura* (9) or triploid *Solanum* (31). In spite of the demonstrated autosyndesis more chromosome numbers were observed near to 13 and 26 than expected in case of a pairing of the 26 chromosomes and a random distribution of the remaining 13. The explanation is supposed to be an irregular distribution of the chromosomes from the polysomes.

6. The described observations are discussed in the light of the results of other workers.

7. The observed autosyndesis between the 13 chromosomes in Asiatic cotton may be the result of either (1) segmental interchange or (2) polyploidy. The polyploid explanation is favoured. Trisomes in a haploid New World cotton (unpublished work) and genetical data (unpublished) confirm the conclusion that the Asiatic cottons ( $n = 13$ ) must be polyploids.

8. The possibility of producing tetraploid Asiatic cotton from the triploid is referred to.

#### IX. ACKNOWLEDGEMENTS.

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## EXPLANATION OF PLATES VIII-XI.

Illustrating Dr. A. Skovsted's paper on 'Cytological Studies in Cotton. I. The Mitosis and the Meiosis in Diploid and Triploid Asiatic Cotton.'

All the figures were drawn with the aid of camera lucida employing a Zeiss oil immersion 1.3 (120  $\times$ ), and ocular 20  $\times$ . All drawings are reduced to 2/3 in the reproduction giving a magnification of about 3,200  $\times$ . The photographs were taken with H. C. Osterstock's cine-camera with oil immersion 1.3 mm. (120  $\times$ ) and ocular 10  $\times$  or 7  $\times$ , giving respectively a magnification of about 1,050  $\times$  and 735  $\times$ .

### PLATE VIII.

Figs. 1-12 are from root-tips. Figs. 13-14 from premeiotic division in the triploid and the diploid. Figs. 15-17 are leptotene from meiosis of diploid *Gossypium arboreum* L.

Fig. 1. Metaphase of diploid *G. arboreum* showing 26 chromosomes, four of which have satellites. A remainder of the nucleolus is visible in the centre.

Fig. 2. Metaphase from the triploid with 39 chromosomes.

Fig. 3. Side-view of anaphase in the diploid.

Figs. 4, 5. Polar view of telophase from the diploid showing fusion of the chromosomes.

Figs. 6-9. Telophase in the diploid demonstrating the formation of prochromosomes and a single nucleolus.

Figs. 10-11. Prophase from the diploid and the triploid showing the tendency to a loosely paired arrangement of the chromosomes. Only the outlines of the nucleoli are indicated.

Figs. 12-14. Late somatic prophase of the diploid and similar stages from the premeiotic division in the triploid and the diploid. Only the outlines of the nucleoli are indicated.

Figs. 15-17. The formation of the leptotene threads from the meiosis of *G. arboreum*.

#### PLATE IX.

Figs. 18-33. Meiosis from the diploid *G. arboreum* or the diploid hybrid (*G. herbaceum* × *G. arboreum*).

Fig. 18. Late leptotene from *G. arboreum* showing condensing of the threads round the nucleolus.

Fig. 19. Zygotene, the doubleness of the thread may be seen at ×.

Fig. 20. Early pachytene showing bivalent strands.

Fig. 21. Pachytene from the diploid hybrid showing about 13 strands terminated by (1) free ends, (2) plasmasomes, or (3) end-to-end conjugation of two strands.

Figs. 22-7. Diplotene stages from the diploid demonstrating the shortening and condensing of the chromosomes. In Fig. 25 the splitting of some of the chromosomes into two chromioles may be seen.

Fig. 28. Polar view from metaphase of *G. arboreum*. The ring-forming chromosome pairs appear as two cylinders when the microscope is focused at their middle.

Fig. 29. Side-view from the metaphase of the interspecific hybrid in which rings and rods may be seen. A single pair is very loosely conjugated.

Fig. 30. Anaphase of the first division.

Figs. 31-3. Interstage showing the reappearing of the split chromosomes.

#### PLATE X.

Figs. 34-5 are from diploid *G. arboreum*. Figs. 36-47 are from the triploid.

Figs. 34-5. Interstage and metaphase from the second division in the diploid showing the split chromosomes.

Fig. 36. Early pachytene from the triploid showing a strand formed of three threads.

Fig. 37. Part of a nucleus in early pachytene. Two strands formed of three threads, several strands formed of two threads, and probably a univalent thread may be seen.

Fig. 38. End-to-end conjugation of two bivalent pachytene strands.

Fig. 39. Diplotene in the triploid. The number of chromosomes in each configuration is indicated by the Roman figures.

Fig. 40. Side-view of all the chromosome configurations in a single pollen mother-cell at metaphase.

Fig. 41. Types of trivalents.

Fig. 42. Types of tetravalents.

Fig. 43. Types of pentavalents.

Fig. 44. Types of hexavalents.

Fig. 45. A septavalent.

Fig. 46. Triploid with a large number of nuclei of different sizes.

Fig. 47. Interstages from the second division in the triploid illustrating one of the 5 per cent. cases in which three nuclei have been formed after the first division. All the chromosomes show splitting.

Figs. 41-66 are from the diploid and Figs. 67-97 are from the triploid. Figs. 61, 62, and 69-71 are taken with ocular 7 ×, the remaining with 10 ×.

#### PLATE XI.

Figs. 48-52. Pachytene in the diploid from the same nucleus as shown in Fig. 21. The doubleness of the pachytene strand and a free end (×) may be seen.

Figs. 53-5. Side-view of metaphase from the diploid with ring-formed bivalents.

Figs. 56-8. Polar view of metaphase in the hybrid showing 13 pairs of chromosomes.

Figs. 59-62. Metaphases from the second division with 13 chromosomes in each plate. Figs. 61, 62 are taken with ocular 7 ×.

Figs. 63-6. Metaphases from the second division in the interspecific hybrid showing 12 + 14 chromosomes.

Figs. 67-8. Trivalent pachytene strand.

Figs. 69-71. Nucleus in the opening stage of the early pachytene. Ocular 7 ×.

Figs. 72-9. Part of the same nucleus as Figs. 69-71 taken with ocular 10 ×. A trivalent pachytene strand with a free end is seen just coming out from the zygotene knot.

Figs. 80-113 are from the triploid and are taken with ocular 10 × except Figs. 108-11 for which ocular 7 × has been used.

Figs. 80-5 are the same nucleus as in Figs. 72-9.

Figs. 86-9. A tetravalent chromosome configuration from diplotene. Each chromosome pair has formed three chiasmata.

Figs. 90-3. A pentavalent configuration of chromosomes from diplotene.

Figs. 94-7. Metaphase from the first division showing from left to right 1 trivalent, 1 bivalent, 1 tetravalent, 1 trivalent, and 2 univalents.

Figs. 98-101. Part of metaphase from first division showing a trivalent, a pentavalent, and a univalent at the end of the pentavalent (best seen in Fig. 98).

Figs. 102-4. A tetravalent between two trivalents from metaphase of the first division.

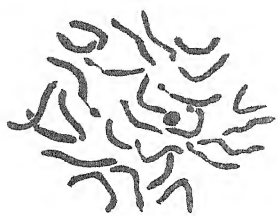
Figs. 105-7. Part of metaphase from the first division showing four trivalents, a univalent, and a string consisting probably of five or six chromosomes.

Figs. 108-11. Metaphase from the second division in the triploid showing 17 + 22 chromosomes. Ocular 7 ×.

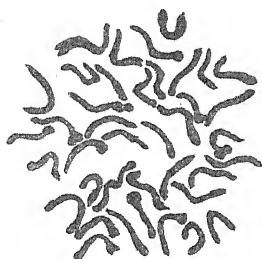
Figs. 112-13. The 22 chromosomed plate from Figs. 108-11 taken with ocular 10 ×. Splitting of some of the chromosomes may be seen faintly.







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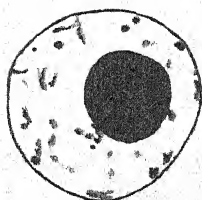
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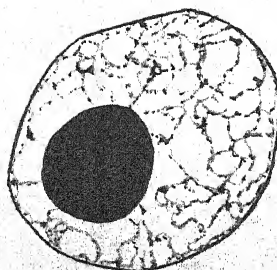
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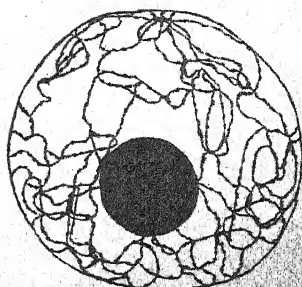
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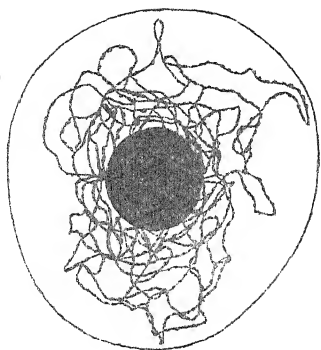
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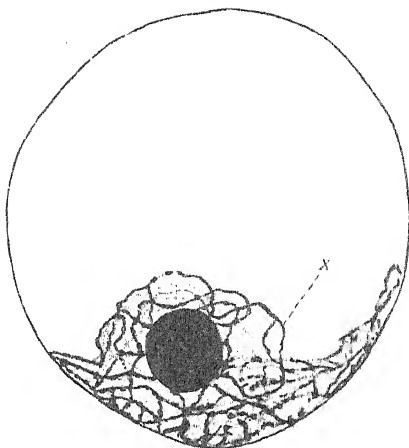
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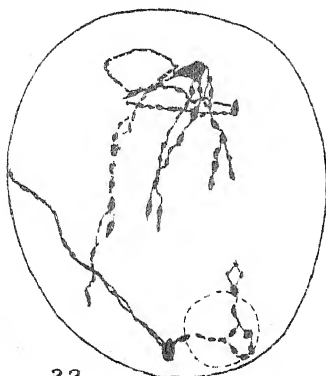
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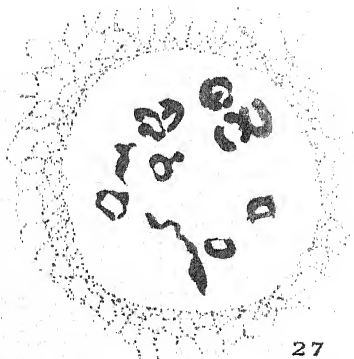
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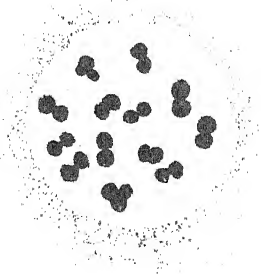
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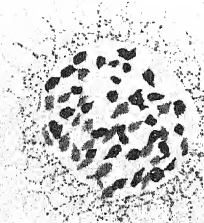
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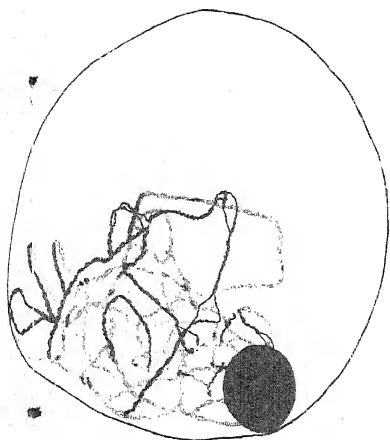
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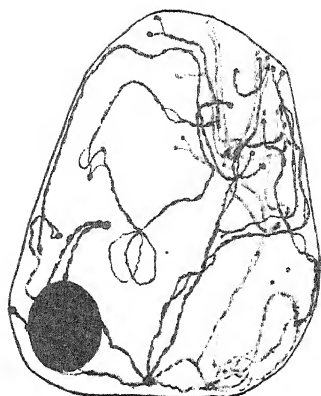
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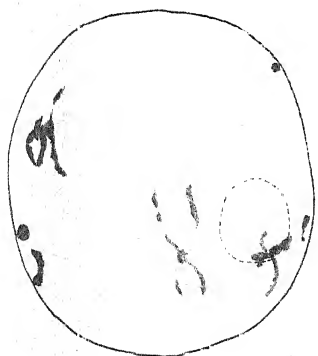
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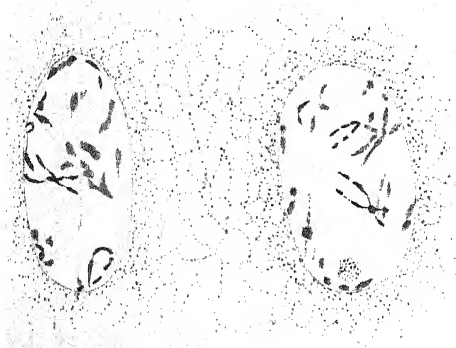
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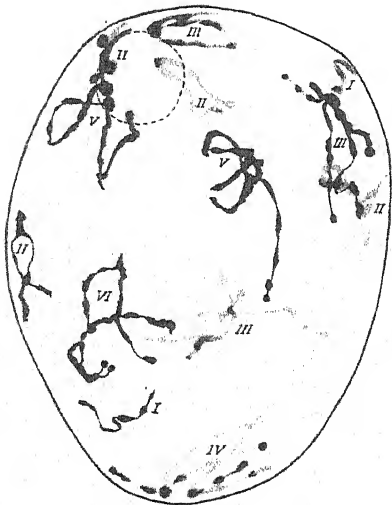
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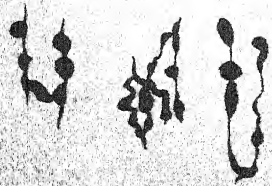
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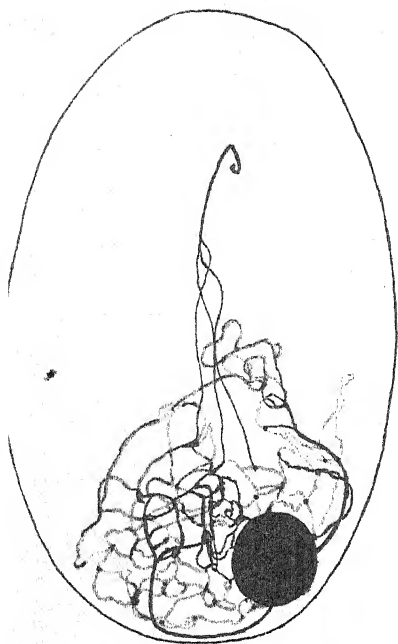


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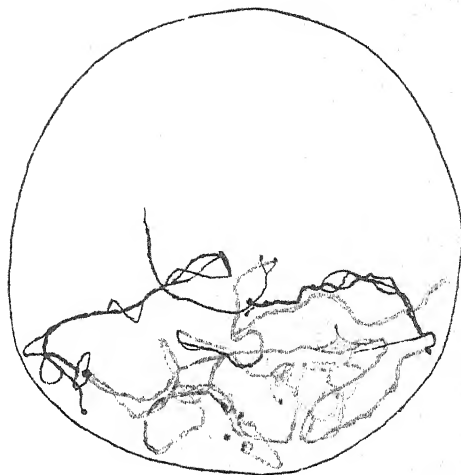


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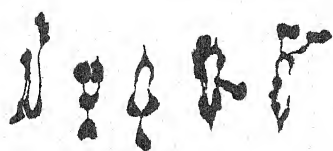
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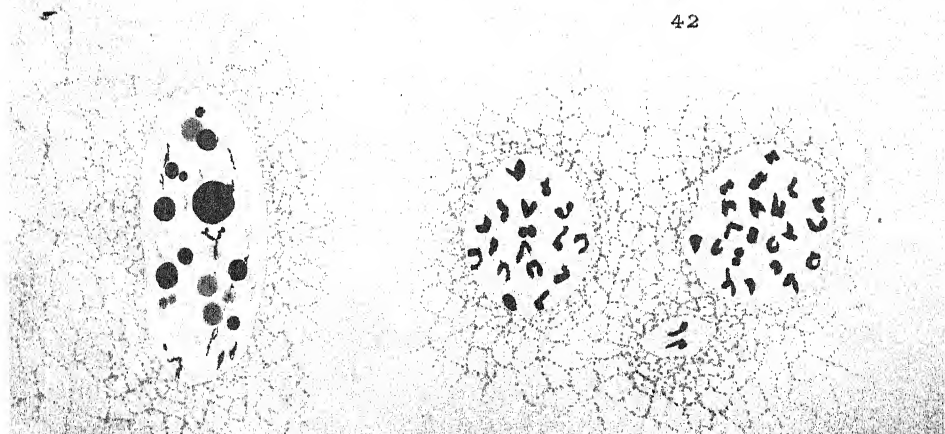
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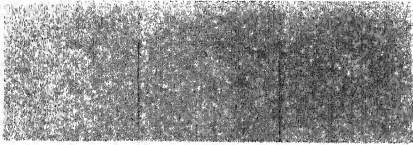


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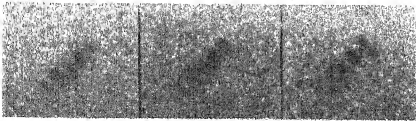


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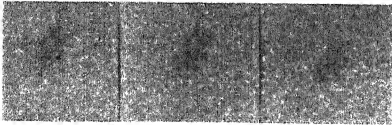




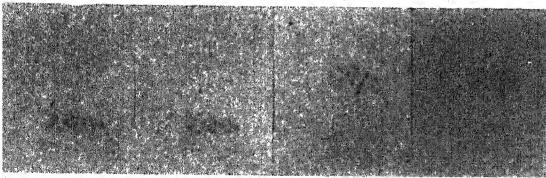
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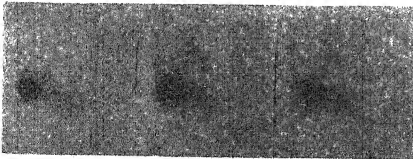
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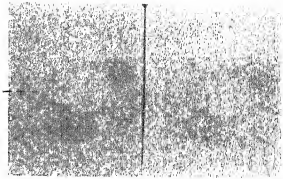
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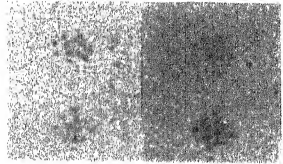
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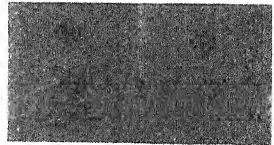
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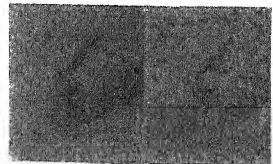
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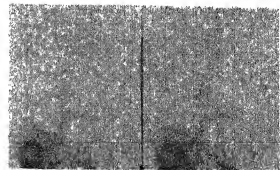
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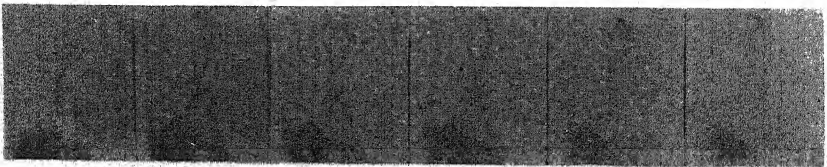
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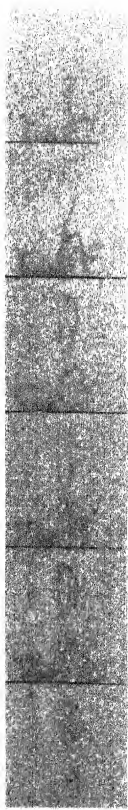


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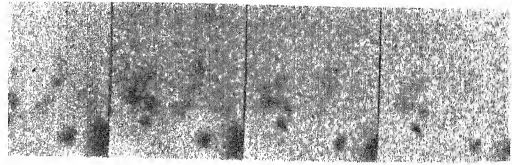
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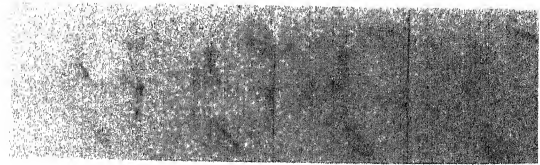


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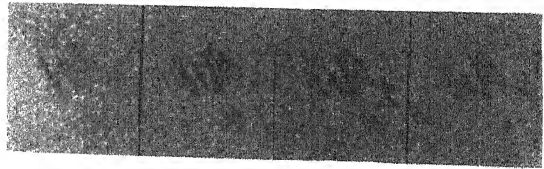


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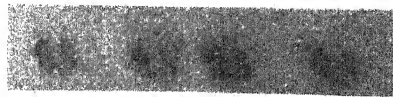


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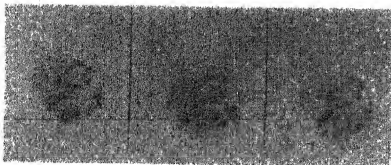


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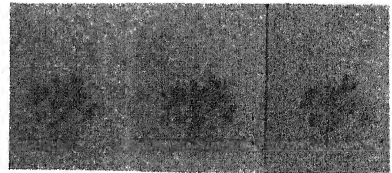
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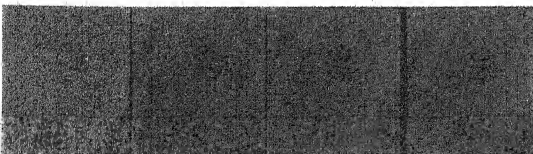
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# Some Marine Algal Balls from Tasmania.

BY

CAROLA I. DICKINSON.

(*Herbarium, Royal Botanic Gardens, Kew.*)

With three Figures in the Text.

IN June 1931 some remarkable algal balls forwarded by Dr. F. A. Rodway, of Nowra, New South Wales, were received at Kew for report as to their nature and origin. Marine algal balls are of such rare occurrence that it appears worth while to give an account of these specimens, and also to outline what is known of other similar bodies which occur both in fresh-water lakes and in the sea.

## *Fresh-water Balls.*

The phenomenon is well known among certain species of *Cladophora* occurring in European fresh-water lakes. As to the mode of origin of these ball forms, Wesenberg-Lund (11) made observations extending over a period of three years on the *Cladophora* formation in Lac Soro, Denmark. He found in one of the creeks of the lake that the balls were formed on the edge of a felt-like growth, where wave action was operative on a rather hard, sandy floor. The plants produced tufted forms by the interlacing of several individuals, and continuous rolling and friction induced a radiating growth and profuse lateral branching, so that the external layers became more and more compact. He comments on the fact that balls are not found among the *Cladophoras* in other Danish lakes, and explains that in the Soro creeks they occur at a depth within the reach of wave motion, at which normally the light intensity would be inimical to growth, but they are there screened by an exceptionally rich plankton from June to March. In April and May, when more light penetrates, the balls become filled with gas (as a result of increased photosynthetic activity?) and then rise to the surface, many no doubt perishing during the two months' exposure.

The Soro balls are about 2-6 cm. in diameter, more or less solid, with a tendency to form a hollow in the centre and cracks radiating from it, due, according to that author, to the production of branches in a lateral

direction, and a consequent tangential increase in the peripheral regions only. As in the *Sphacelaria* balls referred to later, there is a conspicuous concentric arrangement of alternating light and dark zones, which are regarded as annual rings.

Acton (1) has described balls from Lake Kildona in the southern Outer Hebrides composed of *Cladophora holsatica*. Kew specimens from this locality are mere shells of a few millimetres in thickness. There is, however, a distinct zonation, and evidence of centrifugal growth. No. 182 of Areschoug's Alg. Scand. Exsiccatae in the Kew Herbarium is a section from a ball of *C. Sauteri*, and measures 20 cm. in diameter. It is conspicuously zoned and of solid construction, though less compact towards the centre.

### *Salt-water Balls.*

Under marine conditions it is not unusual to find rolled masses of fibre derived from phanerogamic plants such as *Posidonia*. Specimens of these in the Museum at Kew are all much more open in texture, and very different in appearance from the *Cladophora* balls and the *Halopteris* balls described below. The *Posidonia* structures are more often ellipsoidal than spherical, since an elongate piece of the rhizome frequently provides the core. The genus *Posidonia* includes two species, one Mediterranean and the other Australian. According to Sauvageau it occurs at greater depths than any other marine phanerogam (up to 50 m.). The leaves very readily become detached at the ligule, the leaf-sheaths persisting for a considerable time, protecting the new growth. The bases from the oldest upwards become torn to shreds, finally breaking away, to be cast up in quantity on the beach. It is this fibrous material which gives rise to the *Posidonia* balls, bodies of much more common occurrence on the shore than algal balls. The *Posidonia* fibre is so plentiful in some districts that it has been exploited commercially, among other things in the making of paper and mattresses.

Apart from *Cladophoras*, the only notable ball-forming member of the Chlorophyceae is *Valonia aegagropila* Ag., which Kuckuck has kept as a distinct species, but which was considered by Hauck to be a form of *V. utricularis*. Following Kuckuck in keeping it distinct, Boergesen (2) says, 'This species occurs in shallow water, in a locality sheltered by coral reef on the south coast of St. Croix; it was found here abundantly, lying loose on the sandy bottom between sea-grasses. The balls reached here of a size up to a small clenched fist. Furthermore, some few clumps were found in deep water, about 40 m.'

Cases of balling among Rhodophyceae have been alluded to by botanists, but in this group there is nothing which compares with the very compact and truly ball-like forms assumed by the *Cladophoras* and the

two members of the Sphacelariaceae dealt with in this paper. Reinke (6) drew attention to a form of *Furcellaria fastigiata* found in still bays lying loose on the bottom, and growing radially to the size of a man's head. Reinbold (5) recorded it from Kiel, and Lakowitz (4) from the Gulf of Danzig in the Baltic. Species of *Lithothamnium* produce globose forms, and Schroeder (10) mentions *Rytiphloea tinctoria* as a ball-forming alga.

So far as can be ascertained, the only references to really compact marine algal balls concern an *Aegagropila*<sup>1</sup> form of *Sphacelaria cirrosa*. Several writers mention this floating form, but Wittrock (12) is apparently the only one who has published anything as to structure. Specimens found in the region of Ostergarns, off the east coast of Gothland, he describes as of 1–4 cm. in diameter, composed of more or less radiating threads, which by the production of innumerable branches become felted. A section through such balls showed two to three concentric layers each 4–5 mm. thick, each stratum probably representing one year's growth. Nearly all specimens examined, even those of only 1 cm. diameter, had a larger or smaller hollow in the centre. There appeared to be no sandy matrix which could have served as a starting point.

### *The Tasmanian Balls.*

The balls sent to Kew were found during April in a batch of about fifty in number, just above the tidal limit on a sandy beach at Kingston, in Tasmania.

Of the four specimens received, the smallest is  $2\frac{1}{2}$  cm. in diameter, and has a bristly surface, while the largest has a diameter of  $5\frac{1}{2}$  cm. and its exterior is quite smooth. All are nearly spherical, the larger ones flattened a little on four sides, due probably to packing and transport (see Fig. 1).

Attention may first be drawn to the identity of the alga, particularly

<sup>1</sup> The following translation of a paragraph from Schroeder (10) deals with the explanation of the name *Aegagropila*. On account of its general interest it is quoted in full.

‘The first who gives definite information as to these ball-like formations is Olav Worm. Later Linnaeus mentioned in his work appearing in 1763 (*Species Plantarum*, Band II, page 1637) such an alga from the brackish water of the Baltic Sea (*Conferva Aegagropila*). The generic name he borrowed from Pliny, who employed it for the filamentous water plants which were reputed to help to heal broken bones, for the name *Conferva* is derived from the Latin *confervere*, which means ‘to heal together’. In using the species name *Aegagropila* Linnaeus had in mind those balls which occur in the stomach of the Bezoar goat (*Capra aegagrus*) living in Asia Minor and Greece. These consist of dense, compact masses 1–10 cm. in diameter and are commonly called Bezoar stones. In addition such formations are found not only in goats but also in other ruminants, for example, chamois, deer, and camels, and actually those in the last-named animals have often an appearance as to size, form, and colour, which makes them easily mistaken for the sea-balls of *Posidonia*, as I have seen for myself in connexion with such phenomena from camels in the North Sahara and sea-balls formed of *Posidonia* from the Gulf of Naples and East Africa. Linnaeus had therefore chosen a very apt specific name in *Aegagropila*, and Kützing raised it to a generic name for ball-forming *Cladophorae*, in which sense also Kjellmann employed it.’

as the filaments show some interesting features of the Sphacelariaceae, to which group the alga in question belongs. Though the components of the balls are small and fragmentary, representing ultimate parts of much ramified thalli, a number of branches are fructiferous, and it was therefore possible to determine the species forming the bulk of the material. This

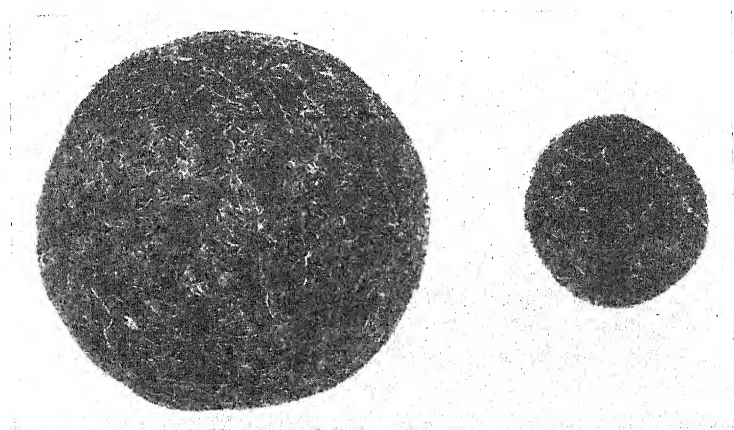


FIG. 1. Balls composed of broken filaments of *Halopteris funicularis* (Mont.) Sauv.  
Natural size.

proved to be *Halopteris funicularis*, a plant originally described by Montagne from Chile, Java, and New Zealand. His description has from time to time been amplified, and a very complete account has been given by Sauvageau, who records it also from Cape Horn, South Africa, Australia, and Tasmania. It belongs to the Holoblastae, a group in which the branches have their origin from the sphacela, occurring therefore at the primary septa. The characters distinguishing *H. funicularis* from those of its immediate allies are the scattered arrangement of the placentae, the long ramified pedicels bearing unilocular sporangia of about  $35\mu$  diameter, and the presence of paraphyses which sometimes become transformed into short pointed ramules (see Fig. 2).

The composition of the *Halopteris* balls may be best ascertained by taking slices about a quarter of an inch in thickness a little to one side of the centre, and teasing them out in water in a shallow dish. All the filaments are under 2 cm. in length, and mostly consist of much ramified branches of definite growth. Among the debris are to be found, somewhat rarely, rather coarser parts of the thallus bearing pericysts. These are large dark-coloured cells from which the rhizoids and adventitious branches (*sensu* Sauvageau) have their origin. In two instances only the pericyst had produced a short rhizoid. There is no inclusion of corticated parts. Nearly all except the short ramules of the last order are either truncate or

have a mutilated appearance. In the latter case they have become dark in colour like the normal apices, and frequently regeneration has taken place by the production of a number of slender filaments from the wound ('pousses de remplacement' of Sauvageau or 'pousses adventives' of Geyler (3)).

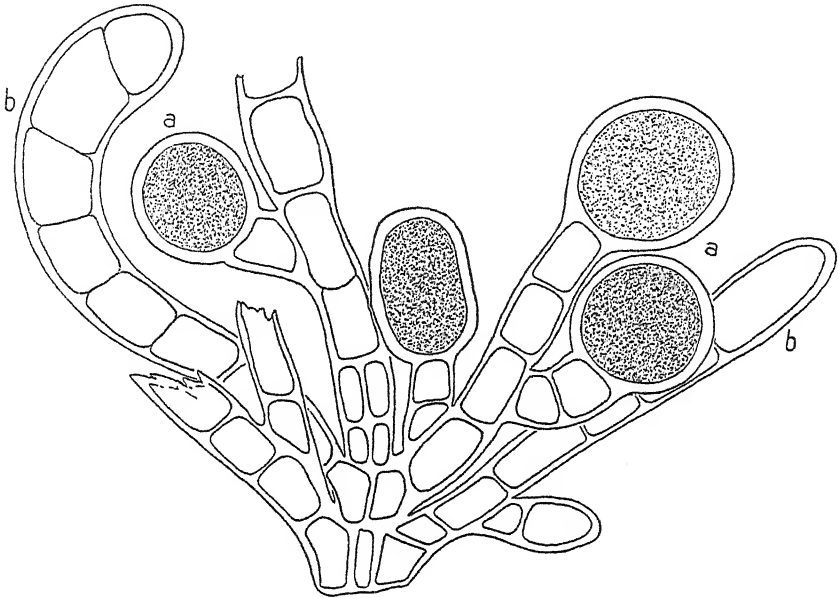


FIG. 2. Portion of a sporangial group of *Halopteris junicularis* from one of the Tasmanian balls showing sporangia (a) and paraphyses (b).  $\times$  about 580.

It is of interest to note also the occurrence of what appear to be plantlets of vegetative origin. These are well-developed shoots of definite growth which have produced a crop of rhizoids from the fracture at the base. They recall the 'boutures' described by Sauvageau (9) in his account of *Stypocaulon scoparia*. According to this author they are as efficient in dispersal and multiplication as the propagules of *Sphacelaria*, but he regards them as accidental and probably due to the ravages of animals, because of the fact that only shoots of definite growth produce plants, those of unlimited growth invariably perishing.

There seems to be no question of the alga taking other than a passive part in ball-formation, since these structures are nothing but an agglomeration of the detached apices of mature fronds, the parts being orientated in all directions. As Sauvageau remarks, rejuvenating growths are by no means uncommon among the *Sphacelariaceae*, and though more frequently encountered in certain species they may appear wherever a fracture occurs at the summit of a vigorous individual. In the specimens under discussion

there is nothing to determine whether such proliferation has taken place before or after the shoots became detached.

Obviously the *Halopteris* balls in their mode of growth are altogether different from those of *Sphacelaria cirrosa*, and are more comparable with



FIG. 3. Section of a ball of *Halopteris funicularis*. Natural size.

the balls of *Posidonia* fibre. Their accrescence is purely mechanical and comes about by the accumulation of plant debris round a core of extraneous matter such as small shells and the skeletons of polyps (see Fig. 3).

As the balls are almost entirely made up of one species they must have their origin either in the *Halopteris* beds themselves, or in some spot in the immediate neighbourhood where the debris tends to accumulate. So long as the growth is continuous, presumably they might attain to large dimensions, but it is probable that they are washed away from the original source of material at an early stage, and as a result of the rolling action of the waves and consequent pressure on the balls the outermost filaments arrange themselves tangentially, giving a remarkably smooth exterior, which no longer affords an anchorage.

#### SUMMARY.

1. An account is given of previous work dealing with ball-formation among species of *Cladophora* occurring in fresh-water lakes, and also of the balls formed by the marine phanerogam *Posidonia*. Their origin is explained and contrasted.

2. The mode of formation of the balls formed from *Sphacelaria cirrosa* as described by Wittrock is summarized and discussed, and it is shown to be similar to that of the fresh-water *Cladophoras*.

3. An account is given of some marine algal balls from Tasmania. These proved to be composed of the alga *Halopteris funicularis*, a member

of the Sphacelariaceae. The structure of these balls and the morphology of the alga concerned is described. Their mode of origin is comparable with that of the Posidonia balls.

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# Development of the Embryo-sac in *Phoradendron*.

BY

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With twenty Figures in the Text.

TWO species of the dioecious genus, *Phoradendron*, are found in abundance in the immediate vicinity of Redlands, California, namely, *P. flavescens* Nutt. var. *macrophyllum* Engelm. and *P. villosum* Nutt. The former species is parasitic chiefly on the cottonwood, *Populus fremonti* Wats., the latter on the live oak, *Quercus agrifolia* Nee. These formed the basis of investigation in the preparation of this paper.

Both species are well represented by an abundance of carpellate and staminate plants in close association. A superficial examination indicates the occurrence of pollination, for while many fruits mature, many others drop off after anthesis, suggesting failure in fertilization. Both types of flowers are borne on short branches which are marked by what appear to be alternate fertile and sterile segments, these imparting a jointed appearance. The fertile segments are the internodes. A longitudinal section through a young carpellate branch shows a flower taking its origin under a stem-encircling collar of tissue that represents connate bracts (Fig. 1). It does not arise in the axil of a bract, but as York (12) found in *Dendrophthora*, on the periblem of the floral axis, a short distance above the axil. Each flower originates by the differentiation of the three perianth lobes, the tips of which meet near the centre over a flat central area, which, because of failure to grow commensurate with the general growth in diameter of the inflorescence axis, becomes sunken in the axis. The outer portion of the perianth lobes is nearly flush with the external surface of the branch. They become laterally separated from the periblem by cleavage furrows extending in from the surface, thus marking at the same time the boundaries of the flower. The flat central tissue constitutes the apex of the axis of the individual flower. By elongation of the internode the first flower forming beneath the bract is carried outward from under it, while a second flower originates underneath, near its axil but above it, in a

manner similar to the first flower. As elongation of the internode continues, the second flower follows the first from beneath the bract, a third



FIG. 1. *P. flavescens* var. *macrophyllum*.  $\times 60$ . Diagram through a portion of the inflorescence apex, showing a young flower under a bract.

starting where the first two did. As many as four flowers in a row may thus emerge from underneath the bract and open up for pollination.

After the perianth and apex of the floral axis become differentiated, further development consists in a widening of the surface of the floral apex which is at first plane, but shortly is seen to project itself into three lobes when observed in a median section parallel to the inflorescence axis (Fig. 2). All three regions enlarge outwards, under the perianth, but the outer grow much faster than the middle section, and finally, by an enlargement toward the centre, approach over the floral apex. These are the carpels (Fig. 3). The



FIG. 2. *P. flavescens* var. *macrophyllum*.  $\times 140$ . Primordia of carpels and placenta.

central mound of tissue which represents the floral apex is at first a flat-topped, short ridge, but it soon becomes elevated near the ends of the ridge, forming two rounded peaks with a depression between (Fig. 3). A cross-section shows this tissue to be longer in the direction of the long axis of the inflorescence, and is of course nothing else than the 'mamelon' or nipple of Treub (10, 11), the 'ovarian papilla' of Johnson (5), and the 'free central placenta' of Goebel (4). Comment on its morphological interpretation has been characteristic of nearly all writers working with the species of Loranthaceae possessing such a structure. As York (12) has given an excellent review of the literature up to the time of his paper (1913), it will be superfluous to repeat it here. More recent work has added no new interpretation, the writers accepting some former explanation, or else adopting a non-committal term such as nipple or papilla, for example, Thoday and Johnson (9), and Dowding (3). Treub (10) and Goebel (4) consider the structure a placental extension of the floral axis, on which the

ovules are reduced to mere archesporia. Goebel (4) uses the expression, 'free central placenta bearing ategminous ovules', and as the structure in *Phoradendron* seems to fulfil this description, the word placenta will be used in referring to it.

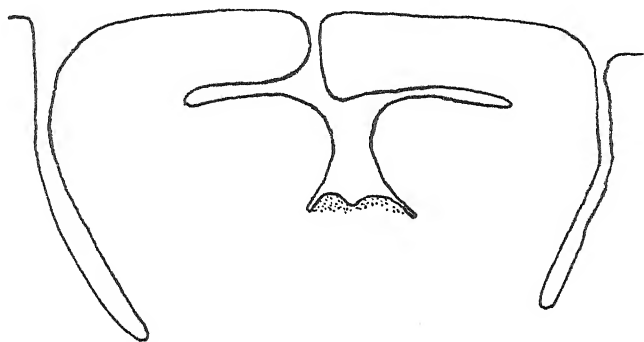


FIG. 3. *P. villosum*.  $\times 100$ . Longitudinal section through flower with very young placenta, showing origin of the two nucellus-like projections (stippled).

*Phoradendron* differs from the other species of Loranthaceae that possess a free central placenta, in that instead of developing it as a simple mound of tissue, or a ridge with a flat or convex top, there are two well-marked rounded projections that correspond to the number of carpels, and arise much as do ordinary nucelli, but without the accompaniment of integuments. They resemble nucelli too, in that each projection develops hypodermally one archesporial cell that takes its origin and becomes distinguishable from surrounding cells at the time cell-elongation within the placenta initiates the protuberances (Fig. 4). There is probably a greater or less coalescence of the inner sides of each nucellus with the floral apex or placenta, which may account for the archesporial cell not lying central in a symmetrically cylindrical or hemispherical tissue. A ridge of tissue becoming two to three times as long as wide must comprise more than a floral axis apex, the form of which would be expected to be approximately hemispherical or dome-shaped. Hence it is reasonable to conclude that the conception of a combination of axis apex and placenta is correct, and that any protuberance bearing an archesporium which may arise on it might logically be considered the nucellus portion of an ategminous ovule. In *Dendrophthora*, two protuberances are found near the base of the placenta. York considers the tissue that composes them as partly nucellus, with the chalazal end near the tip of the placenta and the micropylar portion near the base. In *Arceuthobium* (5), no nucellar protuberances appear to be present. The same is true in *Loranthus sphaerocarpus* in which Treub (11) found a lateral fusion of the placenta with the carpel in three or four places, the intervening free portions representing the ovules

in which the embryo-sacs developed. The greatest reduction is found in *Viscum album* (6), *V. articulatum* (10), and *Loranthus pentandrus* (11) in which practically no axial or placental proliferation occurs, the embryo-

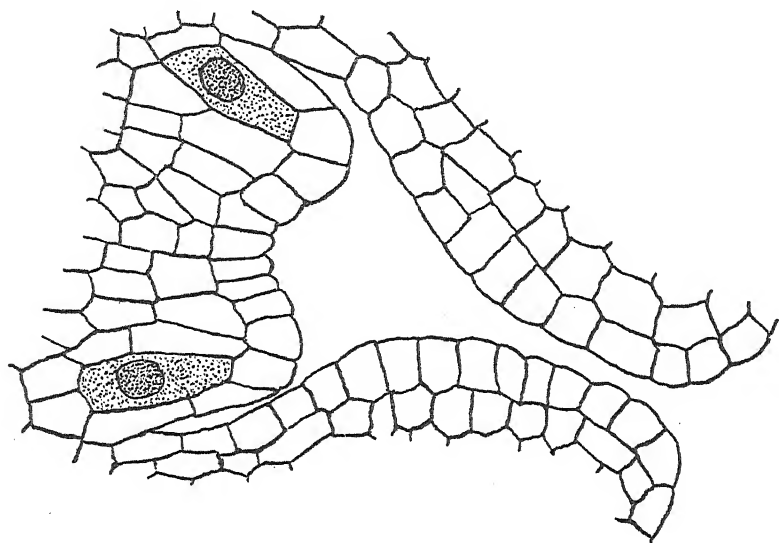


FIG. 4. *P. flavescens* var. *macrophyllum*.  $\times 260$ . Nucelli containing young archesporial cells. Carpels nearly in contact.

sacs developing in the floor of the ovarian cavity. In *Phoradendron*, *Arceuthobium oxycedri* (5), *A. pusillum* (9), *A. americanum* (3), and *Dendrophthora* (12), the placenta is not fused with the walls of the ovarian cavity except at the base, but lies free within it though growing into close proximity to it. As to the size of the placenta in *Phoradendron*, it appears small when compared with that of other genera in which such a structure is found, and except for the archesporial cells, there is no cellular differentiation.

The archesporial cells enlarge, especially in width, and, because of their relatively large size and rich contents, become very conspicuous in comparison with surrounding cells (Fig. 5). They constitute a larger proportion of the placenta than appears to be the case in other genera of Loranthaceae. A cross-section in the stage seen in Fig. 5 is shown in Fig. 6, where it appears as a ridge which is slightly constricted near the centre. The degree of constriction, however, is variable, and may amount to nothing. Cross-sections in *Arceuthobium* (9) and *Dendrophthora* (12) show less difference between length and width in the placenta than is the case in *Phoradendron*, but the height in those genera is relatively much greater.

# DEVELOPMENT OF THE FEMALE GAMETOPHYTE.

The entire archesporium in any single flower of *Phoradendron*, as indicated above, consists of two archesporial cells on opposite ends of the placental ridge. The division of each cell is the first division in the formation of a female gametophyte, hence there are no true megaspores,

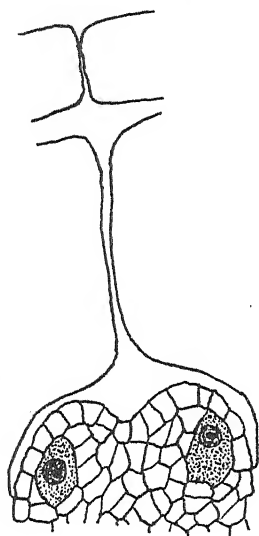


FIG. 5.

FIG. 5. *P. villosum*.  $\times 100$ . Longitudinal section of placenta. Archesporial cells of maximum size and just before heterotypic division. Carpels and perianth lobes seen above.

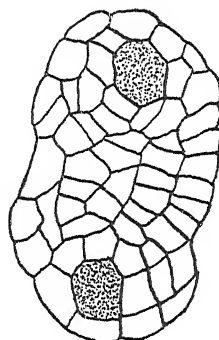


FIG. 6.

FIG. 6. *P. villosum*.  $\times 230$ . Cross-section of placenta at archesporial cell stage. Archesporial cells stippled.

but the archesporial cell functions as the embryo-sac mother-cell. A preliminary division into two daughter-cells or megaspores, of which one becomes the embryo-sac mother-cell, is reported for *Dendrophthora opuntoides* by York (12), and for *Arceuthobium oxycedri* by Johnson (5). Thoday and Johnson (9) think the single hypodermal cells in *A. pusillum* are embryo-sac mother-cells, and hence undergo no preliminary division. Four archesporial groups are to be found within the placental tissue in *A. americanum* according to Dowding (3).

The division of the archesporial cell in *Phoradendron* is heterotypic, the chromosome bivalents appearing on the metaphase plate numbering ten (Fig. 7). The somatic number of the carpellate plant is twenty, and as ascertained by the writer (1) the number for the staminate is twenty-one. The odd chromosome is thus in the staminate plant and apparently determines its staminate character. As absence of it is a carpellate-determining feature, the chromosome sex mechanism of *Phoradendron* may be expressed

as XO-OO (Billings (1)). According to some recent interpretation of sex inheritance, each individual has transmitted to it both male and female

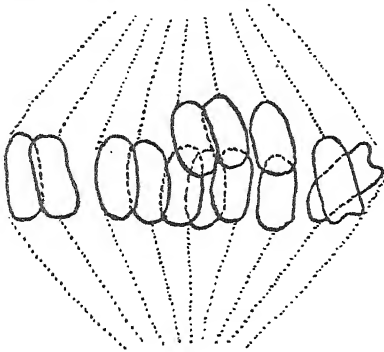


FIG. 7. *P. villosum*.  $\times 1,800$ . Heterotypic division of the embryo-sac mother-cell. Ten chromosomes are on the metaphase plate. Separation of daughter chromosomes is beginning in three instances.

potentialities, the factors of which may be associated with one or more pairs of autosomes. But in a dioecious species a second set of factors may exist which would be connected with some type of sex-determining chromosome mechanism. The function of the mechanism in any individual plant would be to call forth into expression one or the other sex potentiality, the one not so called forth being latent. In plants of the *Phoradendron* type it would appear that the staminate or male-determining factors are associated with the X chromosome that becomes

a particular part of the chromosome complement of the staminate plant; while the carpellate or female determining factors probably lie connected with some autosome.

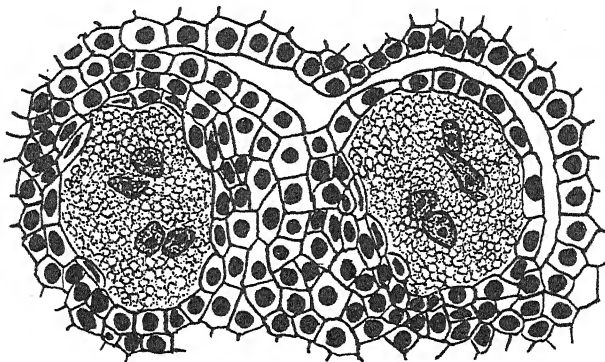


FIG. 8. *P. villosum*.  $\times 230$ . Placenta in longitudinal section, showing the two embryo-sacs contained within it, both in the four-celled stage. Much starch is present.

Only one heterotypic division was observed, so that no reliable report is possible on the amount or character of extruded chromatin, if any. The chromosomes were found to be short and thick in contrast to the longer ones seen in later divisions. The direction of the spindle in the heterotypic division was parallel to the long axis of the placenta, so that the daughter nuclei took the same direction. A similar arrangement is apparently found in *Dendrophthora* according to one of York's figures.

*Phoradendron* belongs to the *Lilium* type of embryo-sac development in the main, though the origin of the primary endosperm nucleus is different.

According to this type (8) the embryo-sac mother-cell undergoes a heterotypic division, forming two nuclei, one of which, the micropylar, gives rise to the egg apparatus and one of the polars, the other, the chalazal, forming

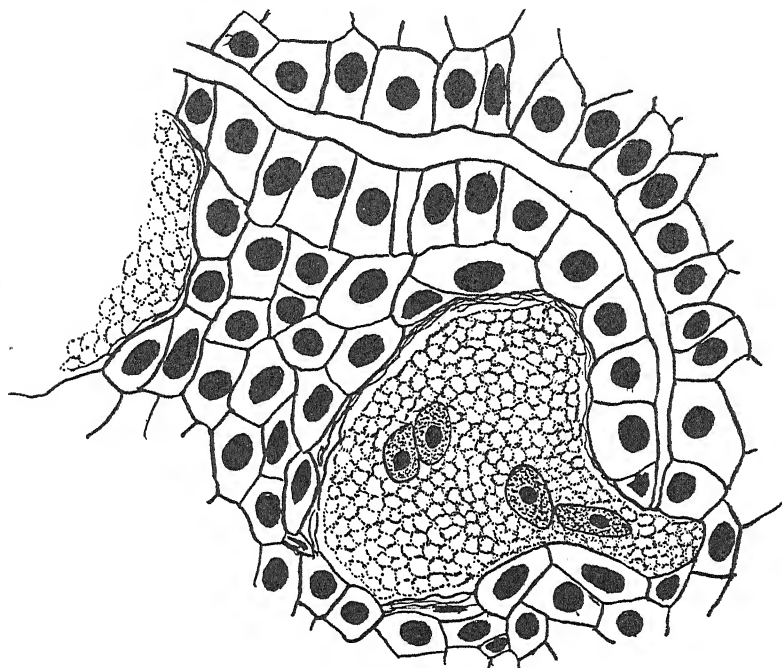


Fig. 9. *P. villosum*.  $\times 650$ . Intra-placental embryo-sac with four nuclei, two of which are about to enter a young haustorium-like long arm.

the antipodals and the other polar. All the nuclei formed by the embryo-sac mother-cell become constituent parts of the mature sac. Divisions by means of which a four-celled sac was created, were observed in *P. villosum*. Ten chromosomes were found passing to each pole, thus confirming the count made on the heterotypic division. Much starch is often stored within the embryo-sacs during this part of their development (Fig. 8), the grains sometimes pressing against the nuclei so as to impart an irregular, even fimbriate, margin.

While the stages described above are taking place within the placenta, the carpels grow together over it, fusing completely, and forming the short style.

After completion of the four-nucleate stage within the placenta, each embryo-sac develops what appears to be a haustorial outgrowth, tubular in form, that passes at first laterally outwards, or else downwards, the direction depending on the depth of the space between the placental base and the carpels (Fig. 9). After passing under this space, the growth turns sharply upwards within the tissue of the carpel. Two of the four nuclei, (probably

sister nuclei), together with some of the starch, pass out of the placenta with the growth, the nuclei keeping just behind the tip. The growth with

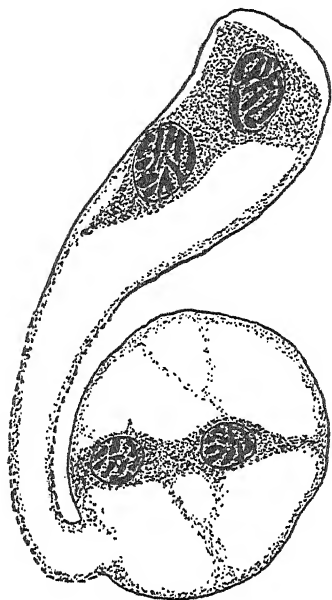


FIG. 10. *P. flavescens* var. *macrophyllum*.  $\times 430$ . Long arm of embryo-sac with micropylar and chalazal nuclei just before division. Binucleate short arm.

its two nuclei continues in an upward direction for a time, and then bends inwardly so as to end in the carpellary tissue almost directly over the portion of the placenta from which it came (Fig. 10). The upper end widens somewhat, and here the nuclei halt their advance. The nucleus that precedes takes a position near the end of the growth, and will be designated as the micropylar nucleus, while the other will for convenience be called the chalazal nucleus. The micropylar nucleus now divides and soon four daughter nuclei are formed. The chalazal nucleus gives rise to two daughter nuclei which keep apart from the other four (Fig. 11). Thus the outgrowth may come to have six nuclei, which with the two remaining behind in the placental portion of the embryo-sac, give to the sac its customary eight. Occasionally, however, the two placental nuclei may give rise to two more.

The four nuclei resulting from the division of the micropylar nucleus at the end of the 'long arm' of the embryo-sac (to use York's designation of a similar outgrowth in *Dendrophthora*) organize to form the egg apparatus which consists of three synergids and the egg. While there is not, perhaps, the degree of organization in *Phoradendron* that obtains in the egg apparatus of many plants, the synergids may take on a pyriform shape, with the egg swung under them (Fig. 11). Three synergids in an egg apparatus is by no means an innovation, though doubtless the occurrence is unusual. York reports three as common in *Dendrophthora*. Dahlgren (2) gives three for *Armeria alpina* and *A. plantaginea*. The same number occur in *Allium odorum* according to Modilewski (7). In all these species, except *Dendrophthora*, however, there is an excess of eight nuclei in the embryo-sac. Other investigators have found more than three nuclei in the egg apparatus, but there was more than one egg. The probable explanation of the presence of the extra synergid in *Phoradendron* is to be found in the origin of the endosperm nucleus, which in its formation does not require the migration of one of the four micropylar nuclei to serve as a polar nucleus, which in typical embryo-sacs would be expected to unite with one from the antipodal group. The origin of the endosperm nucleus in



*Phoradendron* is related to the chalazal nucleus of the long arm. This forms two daughter nuclei that tend to remain in close proximity to each other. Some embryo-sacs, however, in the mature state, contain an undivided chalazal nucleus, this being true even when the other embryo-sac in

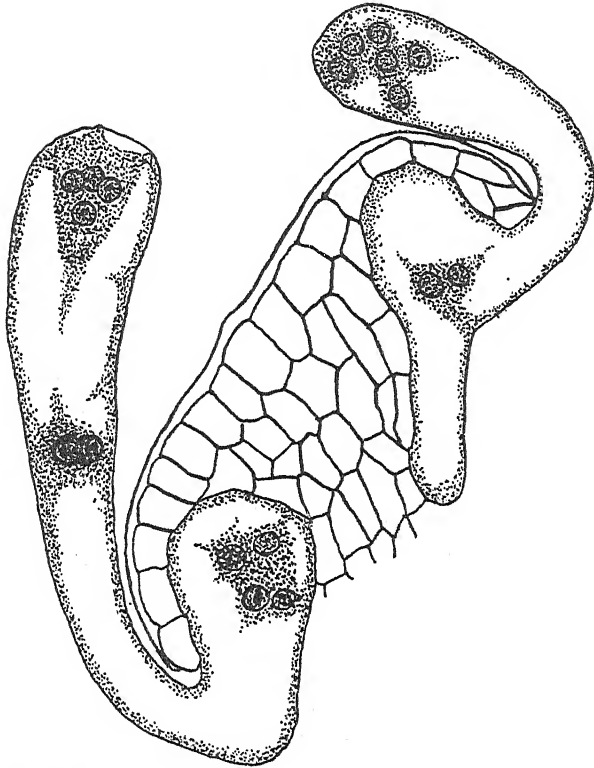


FIG. 11. *P. flavescens* var. *macrophyllum*.  $\times 240$ . Placenta and embryo-sacs. The egg apparatus is organized in one of them. Semi-diagrammatic.

the same flower may show it in the divided state (Figs. 12, 13). It is the chalazal nucleus, divided or undivided, that receives the second sperm in fertilization. The primary antipodal group does not contribute a polar nucleus, which possibly may be due to the relatively remote position of the other polar. Normal double fertilization apparently occurs, though but two instances of it were actually observed. Pollen-tubes have been found passing down the style and carpellary tissue, and entering the long arm of the embryo-sac. In one case the egg and a single chalazal nucleus were receiving their respective sperms; in the other there was a divided chalazal nucleus and a triple fusion to form the primary endosperm nucleus (Fig. 14). It is probable that division of the chalazal nucleus occurs in functional embryo-sacs, and that any single nucleus with which the sperm unites

represents the fused daughter-cells of the original chalazal nucleus, else the endosperm would be diploid at times, and at other times triploid. Chromosome counts of dividing endosperm nuclei indicate their triploid nature.

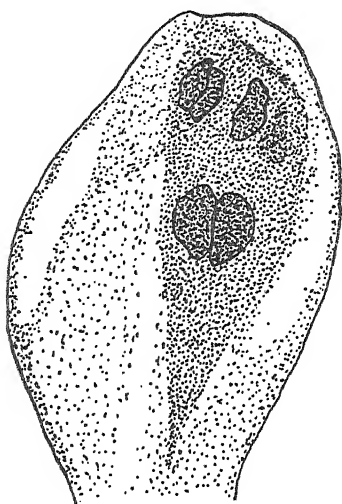


FIG. 12.

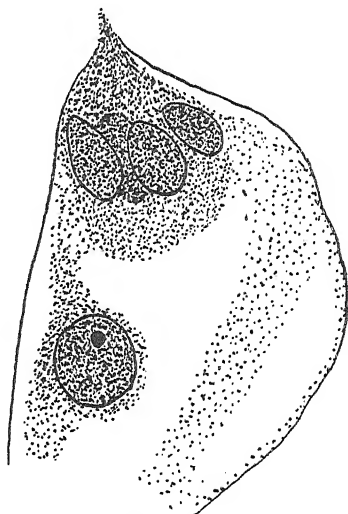


FIG. 13.

FIG. 12. *P. flavescens* var. *macrophyllum*.  $\times 650$ . Egg apparatus and 'polars'.

FIG. 13. *P. flavescens* var. *macrophyllum*.  $\times 650$ . Egg apparatus. Single nucleus in the position of the 'polars'.

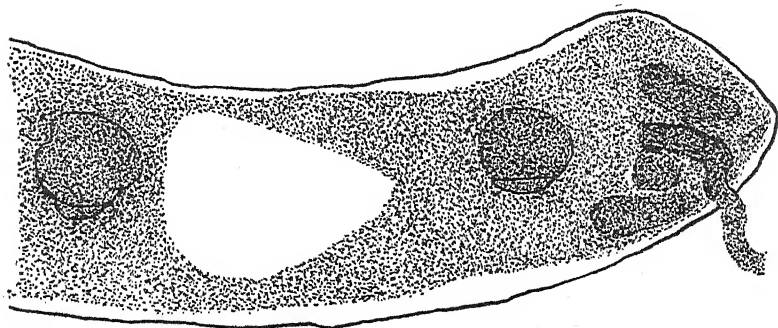


FIG. 14. *P. flavescens* var. *macrophyllum*.  $\times 650$ . Double fertilization with a triple fusion.

Locating pollen-tubes within the carpellary tissue on their way to the embryo-sac should be an easy matter if fertilization regularly occurs, but many flowers show no pollen-tubes even though endosperm is an early stage of development. Explanation probably lies in their rapid disappearance after fertilization has occurred. The period during which pollination may occur is prolonged, probably because of the more or less uncertainty in a wind-pollinated dioecious plant that produces but little

pollen. No particular time can be selected at which pollen-tubes may be expected, and hence the chances of finding them is rendered the more difficult. It is of interest to note that fertilization occurs, not within the

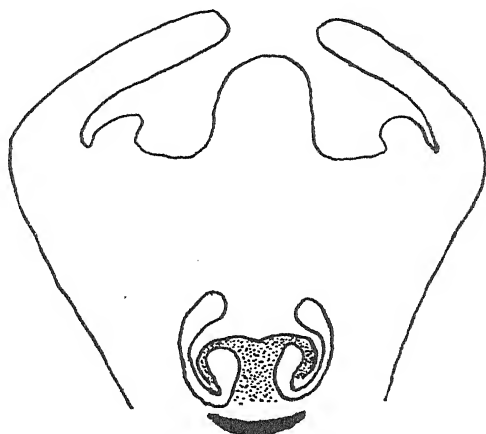


FIG. 15. *P. flavescens* var. *macrophyllum*.  $\times 50$ . Diagram of a longitudinal section of flower, showing the two embryo-sacs about the time of fertilization. Two stamen rudiments are seen near the style. Tracheide plate near base of placenta (black). Placenta stippled.

placenta or ovular tissue, but in the carpellary tissue into which the egg and accompanying nuclei have been conducted by the long arm extension of the embryo-sac.

The two long arms of the embryo-sacs in any particular flower lie in a plane parallel to the long axis of the inflorescence, hence one is more distally located than the other. An endeavour was made to ascertain which becomes the functional sac in endosperm and embryo development. No rule can be established however, as endosperm appeared in either distal or proximal sacs, even among flowers in close proximity. In *Dendrophthora*, York states that the more distally located embryo-sac is the functional one. Both long arms in a *Phoradendron* flower may develop fairly equally, or one arm may be shorter than the other. Occasionally, though rarely, one long arm may suffer collapse before maturity.

The two nuclei remaining in the short arm generally remain undivided, but occasionally divide once each. These nuclei, two or four, probably represent antipodals, since they are the descendants of one of the two daughter nuclei that arise from the first division of the embryo-sac mother-cell. There is no evidence that any of the short arm nuclei enter the long arm after the original pair entered at the time of its inception.

Haustoria may develop from the base of the short arm, and even from the base of the long arm where the two arms join. They pass downward into the tissue below the placenta, but do not penetrate beyond the plate of tracheides that lies near the base of the flower (Fig. 13).

A description of the pistillate flower at the time of fertilization may now be undertaken. The three perianth lobes have a vascular branch each. The outer surface and the edge of each lobe are very heavily

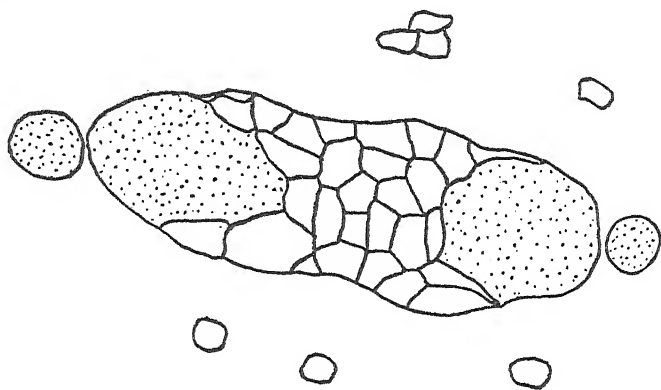


FIG. 16. *P. villosum*.  $\times 230$ . Cross-section of placenta which contained short arms. Long arms just outside at ends. Two vascular strands on one side and three on the other.

cutinized, the cutin layer measuring over  $13\mu$  in thickness. The inner faces are only slightly cutinized. Beneath the perianth lobes in the unopened flower lie the short thick style, and tissue projections that probably represent the stamen rudiments (Fig. 15). The carpels are completely fused. The tissue surrounding the placenta is rich in cell contents, and is supplied by small strands of tracheides that branch off from the main vascular tissue at the base of the flower. There are two to three such small vascular strands opposite each side of the placenta, but none opposite the ends (Fig. 16). A short distance beneath the placenta is the group (mentioned above) of yellow, heavy-walled stone cells or short tracheides which develop earlier in *P. villosum* than in *P. flavescens* var. *macrophyllum* (Fig. 15). The form of this cell mass varies from rectangular to slightly concave on the upper side, at times, however, the concavity becoming pronounced, so as to give the tissue a cup-shaped form. It corresponds to that found in *Loranthus* (11), and in *Dendrophthora* (12). The placenta fills the ovarian cavity, and contains the two short arms of the curved and somewhat C-shaped embryo-sacs (Fig. 16).

When embryo-sac development in *Phoradendron* is compared with that in *Dendrophthora*, as described by York, some remarkably striking resemblances are apparent, chief of which is the peculiar form of the embryo-sacs with their long and short arms, the former in the carpellary, the latter in the placental tissue. It is believed that in no other genera, thus far reported, is such a condition present.

While the general form of the embryo-sac in *Phoradendron* and *Dendrophthora* is similar, there are differences in development and in flower

structure which should be noted. In *Dendrophthora*, the archesporial cell gives rise to two cells, megaspores, one of which becomes the embryo-sac mother-cell. In *Phoradendron* the archesporial cell becomes the embryo-sac mother-cell directly. The placental tissue in *Dendrophthora* is more extensive, at least in height, and is not differentiated so markedly into nucellus-like protuberances. The protuberances in *Dendrophthora*, if such they may be called, are near the base of the placenta, not on the top as in *Phoradendron*. The greater height of the placenta in *Dendrophthora* means a considerable growth straight downward on the part of the long arm of the embryo-sac before the bottom of the ovarian cavity is reached, where the turn outwards and upwards is made. In *Phoradendron* the growth is only slightly, if at all, downward, and may be directly outward because the four-celled stage of the embryo-sac is nearly on a level with the bottom of the ovarian cavity. In *Dendrophthora* the long arm in its growth downwards extends quite to the plate of tracheides underlying the placenta.

The course of development of the embryo-sac from the one-celled to the seven or eight-celled stage in *Phoradendron* differs from that in *Dendrophthora*. The functional portion of the embryo-sac is the long arm, and the nuclei that originate therein are descendants of the two that enter it from the placenta. The divisions that give rise to the descendants occur, in *Phoradendron*, in the upper portion of the long arm, while in *Dendrophthora* they occur chiefly in the embryo-sac before it grows out of the placenta, that is, in that portion of the placenta which later is occupied by the short arm. The antipodals or nuclei remaining within the short arm in the placenta occasionally fuse or partially fuse in *Dendrophthora*. They do not undergo division. In *Phoradendron* they may divide to form four, and were not seen to approach as though attempting to fuse.

The lower or second nucleus to enter the long arm divides in *Dendrophthora* to form what York designates as 'polars'. A similar division of the corresponding nucleus occurs in *Phoradendron*. The propriety of calling these sister nuclei 'polars' may be open to question, because their origin differs from those usually called such. In *Phoradendron*, however, they resemble polars, both as to their location in the embryo-sac, and in their destiny. In *Dendrophthora opuntoides* the polars degenerate, both embryo and endosperm arising from a diploid egg. In *D. gracile* the polars fuse and form a pro-embryo. As no fertilization by an outside sperm occurs, the polar fusion is regarded by York as a kind of fecundation by which the diploid state is restored. In both species of *Dendrophthora* studied by York the endosperm is diploid, but for different causes. In *D. opuntoides* it is diploid because no haploid condition is known to exist, and therefore no fertilization. In *D. gracile* there is a haploid embryo-sac, but as there is no fertilization, the fused polars restore the diploid

chromosome number and originate both embryo and endosperm. In *Phoradendron* the endosperm appears to be triploid.

In both species of *Phoradendron* under investigation, staminate and carpellate plants were found in close association, and true fertilization apparently occurs. The frequent dropping of flowers without the setting of fruits is best explained by failure in fecundation, which in turn can be accounted for by scanty pollen production with wind as the agent of transfer. Apogamy is the rule in the allied genus *Dendrophthora*, but as York found no staminate plants in either *D. opuntioides* or *D. gracile*, the mechanism for maintaining a heterophytic condition in that genus is unnecessary. It would be difficult to account for such a perfect dioecism as that of *Phoradendron villosum* and *P. flavescens* var. *macrophyllum* if apogamy were present, hence fertilization must occur, even though actual observation of it may be difficult.

The long arm of the embryo-sac in both *Phoradendron* and *Dendrophthora* resembles a haustorium. York calls attention to this resemblance, believing that the outgrowth dissolves its way through the carpellary tissue by means of enzymes, in a manner similar to pollen-tubes, and the general run of haustoria. There is every reason to think that the long arm proliferates in the fashion of a true embryo-sac haustorium, even penetrating to richly-protoplasmic tissue provided with vascular connexions. York is correct, however, in not designating the outgrowth in *Dendrophthora* as a haustorium, but by the expression 'long arm' regards it essentially as embryo-sac, or the main part of it, rather than a nutrition-gathering appendage to it. No haustorium, as usually understood, gathers food chiefly for its own development. The short arm of the embryo-sac corresponds to the main portion of the sac in most plants, since it is from this that the haustorium-like growth takes its origin. But as no important structure develops within the short arm, but only in the long one, all food materials absorbed by the latter would be for the benefit of structures within itself, namely endosperm and embryo. Moreover, the long arm conducts with it much of the starch originally found in the intra-placental embryo-sac. For these reasons the haustorial functions of the long arm are secondary to two others, one being that of bringing the egg into a more favourable position for fecundation, the other that of locating a favourable region in which endosperm and embryo are to develop. In instances in which the mature embryo-sac is confined within the placental tissue, as in *Arceuthobium* (5), (3), the growing endosperm enlarges into the overlying carpellary tissue. The long arm extension, as found in *Phoradendron*, may serve the purpose of accelerating development of endosperm by bringing it earlier into a favourable situation.

The purpose of the tracheide plate located a short distance below the base of the placenta is not clear. A similar tissue is found in *Dendro-*

*phthora* (12), and *Loranthus* (10). In the former genus it is similar in form to that in *Phoradendron*, but in *Loranthus* it is deep cup-shaped. In *Phoradendron* the component cells have a pronounced yellow colour, and

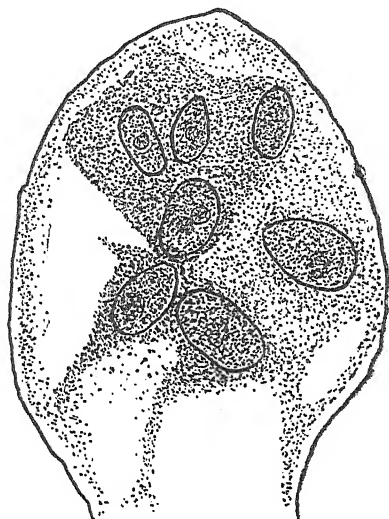


FIG. 17. *P. villosum*.  $\times 650$ . Egg apparatus and young endosperm.

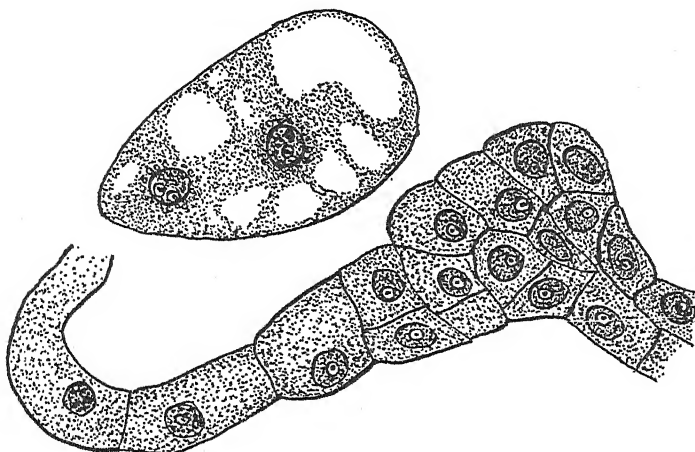


FIG. 18.  $\times 240$ . Endosperm tissue in the base of the long arm of the embryo-sac. Two antipodals with a little starch are seen in the short arm.

are in marked contrast to the tracheides of the vascular strands that enter the flower. They appear to constitute a barrier tissue rather than one for conduction. In *Dendrophthora* York found a considerable number of short vascular strands extending from the upper side of the tracheide plate into the tissue below the placenta. The embryo-sac in its growth downwards

intercepts some of these short strands. In *Phoradendron* there is no vascular tissue directly between the placenta and the tracheide plate. A single vascular strand was found passing upwards towards the bottom of the plate, but as a rule the bundles that enter the base of the flower and

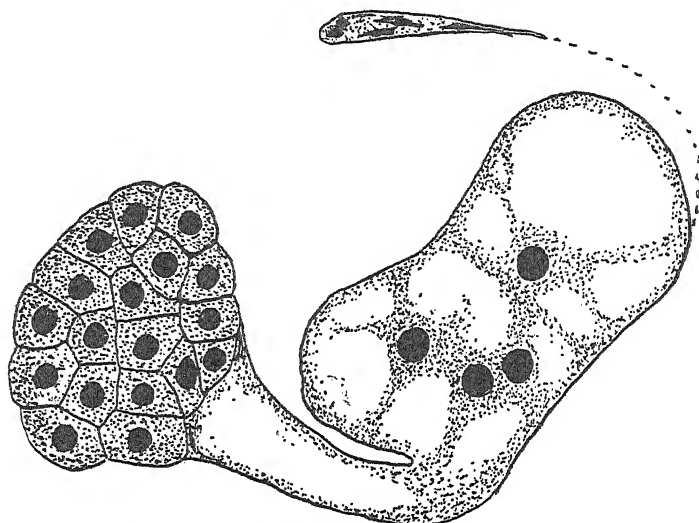


FIG. 19. *P. flavescens* var. *macrophyllum*.  $\times 230$ . Endosperm in expanded part only of the long arm. Coalescence of the short arms. Remains of non-functional embryo-sac still visible.

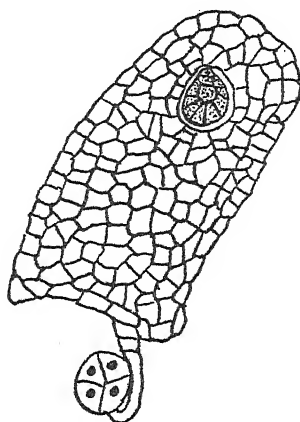


FIG. 20. *P. villosum*.  $\times 60$ . Endosperm in both long and short arms. Embryo not cut in median longitudinal section.

end in the rich carpellary tissue above the placenta, take their course around the plate. When the endosperm has attained a considerable size, the tracheide plate is pushed down, and appears to share the fate of other crushed tissues.



#### EARLY POST-FERTILIZATION DEVELOPMENT.

The zygote remains undivided for a time, during which endosperm develops in the upper end of the long arm of one of the embryo-sacs (Fig. 17). Endosperm nuclei may pass into the empty lower and narrow portion of the long arm, or may stop short at the base of the expanded part (Figs. 18, 19). The zygote and earlier stages in embryo development can be distinguished from the endosperm only with difficulty. The endosperm, which soon becomes a tissue, enlarges upward and outward more rapidly than downward, so that the placental portion of the embryo-sac may stand intact, even after the endosperm has attained considerable size (Fig. 20). An endosperm tissue may form within the short arm of the embryo-sac if one of the nuclei succeeds in reaching it (Fig. 20).

While developments are taking place within the long arm of the embryo-sac, the short arms within the placenta may enlarge to such a degree that they coalesce, the long arm of the non-functional sac collapsing and appearing as a vestige (Fig. 19). Fusion of the short arms is known to occur in *Dendrophthora* (12). A nuclear fusion may also occur in this genus, but none was observed in *Phoradendron*.

#### SUMMARY.

*Phoradendron villosum* and *P. flavescens* var. *macrophyllum* show great similarity in embryo-sac development.

Carpellate flowers originate beneath a collar of tissue representing connate bracts, and emerge one at a time as the internodes elongate.

The two carpels enclose a central ridge of tissue which probably represents a combined placenta and floral axis apex. This is the 'mamelon' of Treub.

The placental ridge gives rise to two rounded tissue projections that resemble nucelli. During elongation of the placental cells to form the projections, or nucelli, a single archesporial cell is differentiated in each nucellus through larger size and richer contents.

Each archesporial cell undergoes a heterotypic division of its nucleus, the resulting nuclei becoming directly a portion of the embryo-sac contents. No true megaspores are produced, the archesporial cell being transformed into the embryo-sac mother-cell.

The female gametophyte is haploid, with ten chromosomes passing to each pole during anaphase. The somatic count in the carpellate is twenty, that in the staminate plant twenty-one. The sex chromosome mechanism may therefore be expressed by the formula XO-OO.

After developing a four-celled stage within the placenta, each embryo-sac sends out a haustorium-like outgrowth which passes beneath the floor of the ovarian cavity, and turns upwards or towards the base of the style,

ending finally in an enlarged apex approximately over the portion of the placenta from which it arose. The outgrowth is not a true haustorium, but the main or functional portion of the embryo-sac. Two of the four nuclei in the embryo-sac within the placenta, with starch, if present, accompany the outgrowth (long arm of the embryo-sac), keeping just back of its tip. The upper of the two nuclei (micropylar nucleus) gives rise to four daughter nuclei that organize as an egg and three synergids. The lower nucleus (chalazal nucleus) may divide to form two 'polar' nuclei. The embryo-sac form, and arrangement of contents, is strikingly similar to that found in *Dendrophthora* as described by York.

Pollen-tubes pass down the style and carpellary tissue to the upper end of the long arm of the embryo-sac. Fecundation of the egg and fusion of one of the sperms with the 'polar' nuclei was observed. It appears to be a matter of indifference which embryo-sac of the two in each flower, distal or proximal with respect to the base of the axis of inflorescence, becomes the functional one.

Endosperm arises from the triple fusion of sperm and 'polars'. Nuclei may find their way to the base of the long arm of the embryo-sac, occasionally into the short arm within the placenta, or may stop near the base of the expanded upper portion of the long arm. Organization into tissue occurs early in development.

The zygote remains undivided until considerable endosperm has been formed. The embryo is without a suspensor.

No monoecious plants or hermaphrodite flowers were observed.

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# Studies of the Physiological Importance of the Mineral Elements in Plants.

## IV. The Quantitative Distribution of Potassium in the Potato Plant.

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With seven Figures in the Text.

### INTRODUCTION.

THE present paper is the last of a series of three that describes a research into the distribution of potassium in the potato plant. The first two parts have already appeared in this journal, and deal with the distribution as revealed by histochemical methods (11), and the quantitative partition between the major organs at different stages of growth as given by gravimetric analysis (5). The work now described consisted of a more detailed quantitative survey of the distribution of potassium, confirming and elaborating the results in the previous papers. In all a more or less complete picture of the distribution at all stages of the growth of this plant has been obtained.

### METHODS.

*Sampling.* Three separate series of samples were taken, one at Islip, Oxon, in the summer of 1928, one in 1931, and a third at Highgate, also in the latter year. In each case potato plants of the variety 'Majestic' were used, and the principle of sampling was always the same. A number of haulms ranging from thirty to forty was taken and divided into pieces each consisting of a node with its leaf, the bud or shoot subtended by the leaf, and the internode below. Each sample was thus made up from thirty or

forty plants, and after removal to the laboratory was dried at 100° C. for 48 hours, ground to a fine powder, and sealed up in a labelled glass tube to await analysis. In the first and third series selected internodes numbered from the apex down the entire length of the stem were examined, including an apical sample comprising the whole of the unfolded terminal bud. In the second series this terminal bud alone was taken and its individual segments carefully dissected from one another and analysed individually. Separation of stem and leaf portions was not achieved in this series, but in the first and third this was done, and in the first the axillary buds and shoots were separated also.

*Potassium analysis.* The potassium in the first series of samples was estimated by the perchlorate method as described by Cumming and Kay (2) and Brown (1) with only minor modifications. This method was also used for the work of the previous paper (5), but while it proved entirely satisfactory for these relatively large samples, it was by no means sensitive enough for the very small quantities involved in series 2. For these the cobalti-nitrite method of Kramer and Tisdall (7) was used as modified by Kerr (6). This is a volumetric micro-method and was found to give satisfactory results down to about 0.005 mg. potassium. It is also considerably less laborious than the perchlorate method and on this account was used for the final set of samples. The principle of Macallum's method used in the histochemical part of this work (11) depends also on the insolubility of potassium cobalti-nitrite, but it is nevertheless a matter of some interest that the three methods employed have led to a consistent picture of the potassium distribution and have qualitatively, at least, agreed one with another.

In each sample the dried material was ashed by cautious heating to a dull redness in a silica or porcelain crucible for six to ten hours. After cooling and weighing, the ash was dissolved in a few drops of concentrated hydrochloric acid and evaporated to dryness on a water bath. The contents of the crucible were then extracted repeatedly with boiling water and filtered into a measuring flask and made up to a suitable volume. With the very small samples of the second series it was found advisable not to filter but merely to allow the insoluble residue to sink to the bottom of the flask, which it did very readily.

#### EXPERIMENTAL RESULTS.

*First series.* Material for this series was collected on five occasions beginning on 27th of June when the stem had just produced its full number of internodes, seventeen on the average, and finishing on the 22nd of August, when the four lowest ranks of leaves had withered and fallen. The primary data obtained, viz. dry weight, weight of ash, and weight of potassium of internodes 1, 2, 4, 8, and 11, and their attached buds and leaves are recorded in Table I.

TABLE I.  
A. Centigrams of Dry Matter, Ash, and Potassium per Plant.

Segment.	27 June.			12 July.			25 July.			8 August.			22 August.		
	Dry wt.	Ash.	Potas- sium.	Dry wt.	Ash.	Potas- sium.	Dry wt.	Ash.	Potas- sium.	Dry wt.	Ash.	Potas- sium.	Dry wt.	Ash.	Potas- sium.
1 Leaves	8.14	0.737	0.283	22.30	2.893	1.090	18.26	2.523	0.742	25.03	3.598	1.042	32.23	5.038	1.320
Stems	3.00	0.459	0.212	12.10	2.015	1.224	10.52	1.865	0.905	13.66	2.459	1.013	16.07	3.223	1.048
Axillaries	5.40	0.501	0.233	10.49	0.862	0.384	4.41	0.408	0.151	3.71	0.323	0.062	0.50	0.034	0.204
2 Leaves	15.71	1.450	0.670	37.25	5.013	1.762	27.83	3.943	1.182	44.60	6.823	2.086	40.98	7.000	1.795
Stems	5.08	1.058	0.489	18.36	4.328	1.900	19.13	4.703	2.012	28.03	6.250	3.150	28.38	7.503	2.125
Axillaries	5.58	0.549	0.250	41.37	5.732	2.692	46.96	7.321	2.909	94.52	15.164	5.214	98.10	15.459	5.102
4 Leaves	22.20	3.118	1.265	46.28	6.848	2.584	50.85	7.070	2.507	64.13	10.200	3.220	69.28	11.900	2.830
Stems	11.06	3.445	1.209	23.81	6.380	2.220	28.00	7.225	3.000	38.63	9.380	3.800	40.30	9.318	3.040
Axillaries	0.48	0.045	0.031	46.28	0.333	0.147	4.43	6.378	0.208	21.05	3.158	1.268	18.86	3.270	1.194
8 Leaves	37.23	5.643	1.718	39.23	6.968	2.114	44.38	7.205	1.858	40.40	7.025	2.000	44.53	8.800	1.845
Stems	23.67	5.718	3.047	34.83	8.133	3.460	39.20	9.770	3.580	40.23	9.213	2.950	44.33	9.865	4.300
Axillaries	1.06	0.139	0.055	2.66	0.411	0.163	2.37	0.396	0.122	3.68	0.596	0.221	4.50	0.803	0.209
11 Leaves	21.84	5.870	1.512	19.30	3.648	0.834	26.60	4.936	1.116	12.43	2.505	0.588	14.42	3.087	0.678
Stems	21.97	5.090	2.304	33.35	6.993	2.213	35.25	7.618	2.684	35.25	6.075	1.685	36.45	7.483	3.070
Axillaries	5.10	0.772	0.322	10.44	1.711	0.674	4.08	0.674	0.213	9.96	1.771	0.656	18.32	3.573	1.086

After the first sampling the terminal bud flowered, and the axillary shoot of the second leaf carried on the growth of the haulm. This axillary was then divided into segments corresponding with internodes and each analysed separately (see Fig. 3).

It will be noticed that the distribution of potassium and total dry matter up the haulm run roughly parallel. In the leaves and stem portions the greatest amounts of potassium and dry matter are contained by the middle members of the haulm; while in the axillary buds there is a minimum in the middle contrasting with the maxima at the lower levels and at the second segment, the latter being the axillary that carries on apical growth after flowering. In the first sample, taken before flowering, this accumulation is faintly foreshadowed.

The reality of this distribution of potassium is demonstrated when the results are examined by Fisher's analysis of variance. The total variation calculated from the data of Table I is 115.8222 and is distributed among 74 degrees of freedom. The partition is shown in Table II together with the significance of each item as given by the 'Z' test (3).

TABLE II.

*Analysis of Variance of Potassium in Samples.*

Source of variance.	Sums of squares of deviations.	Degrees of freedom.	Variance.	Z Found.	for P = 0.05.
Organs . . . . .	21.5977	2	10.7989	1.9167	0.61
Levels . . . . .	22.5800	4	5.6450	1.5923	0.51
Occasion . . . . .	11.2255	4	2.8064	1.2422	0.51
Differentials.					
Organs × levels . . . .	37.8433	8	4.7304	1.5038	0.43
Levels × occasions . .	13.1422	16	0.8214	0.6285	0.39
Organs × occasions . .	1.8265	8	0.2283		
Remainder . . . . .	7.6070	32	0.2377		
Total . . . . .	115.8222	74			

With the exception of the last differential, the interaction between organs and occasions, all the classes of variance reach the conventional level of significance (nineteen chances to one), and most of them considerably exceed it. The data show, therefore, that there are significant differences in the quantities of potassium present at different heights up the stem, that there are also significantly different amounts at the different stages of growth, and in the three types of organ. Further, the significance of the two differential variances means that the differences between organs vary significantly at different levels and that the levels themselves have different time sequences. There is, on the other hand, no evidence that the organs show different relations to one another at different times, the variance due

to organs  $\times$  occasions being indeed very much smaller than any of the others; in other words, the relative amounts in stem, leaves, and buds tend to remain constant.

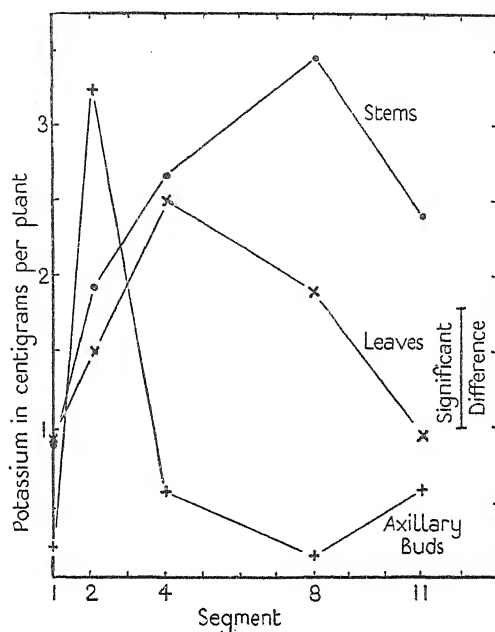


FIG. 1. The average potassium content in centigrams of leaves, axillary buds and stem segments at different levels of the haulm. These curves show the averages of the five occasions of sampling.

In order to get the symmetrical table necessary for the analysis the axillary shoot of the second internode was treated all through as a unit: that is to say, the individual values obtained for the segments of this shoot were ignored and their sum only employed. A good deal of the variation is due to the preponderance of this shoot over the other axillaries, a preponderance that increases rapidly with time. The significance of any given comparison can, however, be tested by determining its standard error, using the remainder variance, 0.2377, as the variance of any single potassium value in Table I. The standard error of any comparison is then given by

$$S.E. = \sqrt{\frac{0.2377 \times 2}{n}}$$

where  $n$  is the number of figures averaged in each of the items compared. Thus for the content of leaves at different levels we have

$$S.E. = \sqrt{\frac{0.2377 \times 2}{5}} = 0.3084.$$

Since  $n$  is not large the value of the comparison also depends on its size, and for the given value of five any difference to be significant must not

be less than  $0.3084 \times 2.571 = 0.7839$  (See Fisher's Table of  $t$ , 3). The same criterion applies to stems and buds. In Fig. 1 the potassium content of each of these 'organs' is set out and the critical value also shown at the side. It will be seen at once that the accumulation of potassium in the middle regions of the stem and its attached leaves is easily significant. As far as the evidence goes, the only axillary that differs from the others is the second; the differences between the remaining dormant buds do not reach significance.

The dry weight of the various organs runs more or less parallel with their potassium content. There is undoubtedly a strong correlation between the two quantities, and it might be supposed that for a given type of organ the amount of potassium was solely a function of its dry weight. This is not, however, entirely true. The simplest way of examining the relation is to use the potassium/dry weight ratio in its familiar form of grm. potassium per 100 grm. dry matter, and to see whether this does in fact remain constant. Table III gives the results of an analysis of variance of this ratio.

TABLE III.

*Analysis of Variance of  $\frac{\text{Potassium} \times 100}{\text{Dry weight}}$ .*

Source of variance.	Sums of squares of deviations.	Degrees of freedom.	Variance.	Found. Z	for $P = 0.05$ .
Organs . . . . .	247.9983	2	123.9992	2.4542	0.61
Levels . . . . .	23.0828	4	5.7707	0.9205	0.51
Occasions . . . . .	7.4501	4	1.8625	0.3552	0.51
Differentials					
Organs $\times$ levels . . .	19.8930	8	2.4866	0.5000	0.43
Levels $\times$ occasions .	21.0558	16	2.6320	0.5264	0.39
Organs $\times$ occasions .	12.9186	8	0.8704		
Remainder . . . . .	29.3004	32	0.9156		
Total . . . . .	361.5930	74			

The results show that the variation between the organs is now greater than before. The variation between different levels is smaller than in the previous analysis but still comfortably significant. There is also evidence that the differences between organs still vary significantly with level, and the differences between levels with time. Applying the 't' test as before, the significant level differences are found to lie principally in the stem: there is still a significant increase in the middle internodes (see Fig. 2), but not in the leaves attached: that is to say, 'storage' occurs in the middle (actually in the upper middle part) of the stem. The top axillary that aborts has a significantly lower percentage than any of the others, but the active second axillary does not differ significantly from the sluggish or completely dormant ones lower down the stem.



In the foregoing paragraphs the axillary shoot of the second segment has been treated as a unit similar to the other axillaries, but by a physiologist it may also be considered as the upper end of the main stem. The whole of the top segment then becomes merely a side branch terminated by a sterile inflorescence. From the latter standpoint it is interesting to notice how the graphs of the potassium contents of its internodes 'fit on' to those of the main stem (Fig. 3). As already stated, the ratio of the potassium to total dry matter agrees with that of the other axillaries rather than with the upper middle regions of the mature stem.

*Second series.* Towards the end of the growing season of 1931, 120 apical buds were collected and divided into four lots of thirty. Each lot was then dissected with a sharp scalpel into apices and four consecutive segments below; each segment consisting of an internode and leaflet. The samples, twenty in all, were at once put into stoppered weighing bottles and the fresh weight obtained. The dry weight was taken after 48 hours drying and the potassium determined quantitatively by the cobalti-nitrite micro-method. These results are given in Table IV.

TABLE IV.  
*Dry Weight, Water Content, and Potassium Content.*

(Centigrams per plant.)			
Segment.	Dry weight.	Water.	Potassium.
Apex	0.017	0.100	0.00097
<i>b</i>	0.373	0.195	0.00096
<i>c</i>	0.189	1.094	0.00426
<i>d</i>	1.250	8.065	0.03234
<i>e</i>	5.306	36.004	0.16758
Apex	0.011	0.060	0.00079
<i>b</i>	0.037	0.186	0.00237
<i>c</i>	0.279	1.495	0.00671
<i>d</i>	1.527	7.509	0.05250
<i>e</i>	6.268	39.082	0.21500
Apex	0.016	0.101	0.00079
<i>b</i>	0.043	0.213	0.00082
<i>c</i>	0.308	1.720	0.00624
<i>d</i>	2.088	11.782	0.02810
<i>e</i>	8.214	49.736	0.29000
Apex	0.013	0.082	0.00149
<i>b</i>	0.023	0.116	0.00135
<i>c</i>	0.177	0.949	0.00322
<i>d</i>	1.351	8.059	0.01948
<i>e</i>	6.268	46.322	0.30700

It will be noticed that the actual amount of potassium in each internode does increase with growth, but its ratio to the dry matter at first drops and then begins to rise again. The increase of potassium, that is to

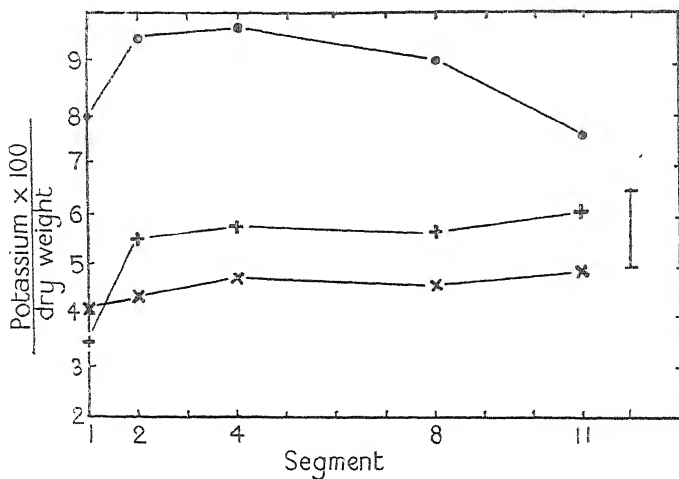


FIG. 2. The average  $\frac{\text{potassium} \times 100}{\text{dry weight}}$  ratio for leaves, axillary buds and stem segments at different levels of the haulm. These curves like those of Fig. 1, show the averages of the five occasions of sampling.

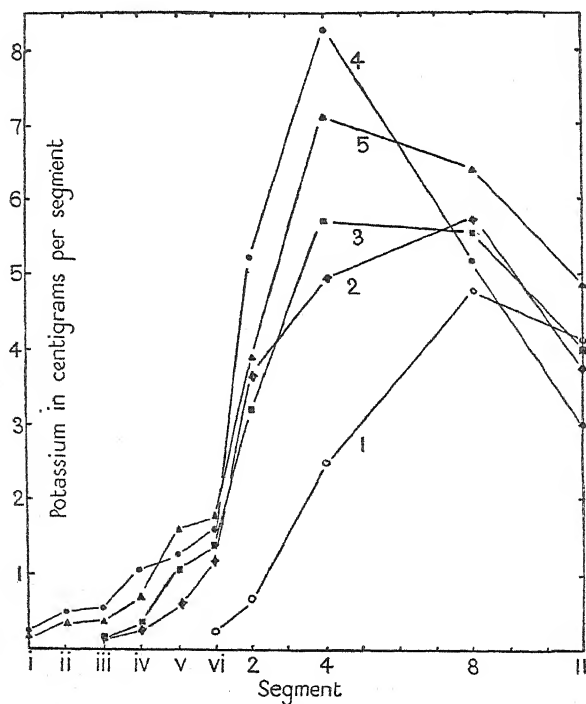


FIG. 3. Centigrams of potassium in the segments (leaf + bud + internode) up the haulm and its main axillary shoot. The numbers show the order of sampling: 1 = June 27; 2 = July 12; 3 = July 25; 4 = August 8; 5 = August 22.

say, does not keep pace with the total accumulation of dry matter for a time but a little later outstrips it: there is also a similar relation towards the amount of water present. The significance of these comparisons was tested by analyses of variance, the results of which are given below.

TABLE V.  
*Variances of Potassium Content.*

	Potassium per segment.	Potassium $\times$ 100 dry weight.	Potassium $\times$ 100 water.
Levels . . . . .	0.0597	7.3316	0.4923
Samples . . . . .	0.0005	5.7798	0.2070
Remainder . . . . .	0.0010	2.1894	0.0689
Significant difference between levels	0.0622	2.9065	0.5158

There is significant variation between the different segments in each method of comparison and by comparing the 'significant differences' with the curves of Fig. 4, it will be seen that the dips in the percentages relating

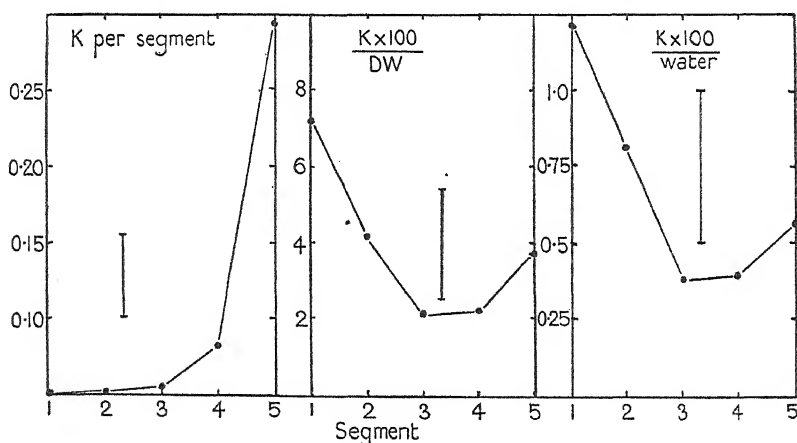


FIG. 4. The average content of potassium per segment expressed as weight per segment, percentage of dry weight, and percentage of water weight. The magnitude of the significant difference is shown by the vertical line in each case. In addition to the dip from the apex, the beginning of the rise towards the middle of the stem is indicated.

potassium to dry weight and water are significant. The differences between samples, though rather large, do not reach significance, and since the samples were fairly big, involving thirty plants apiece, this might reasonably be expected.

The most interesting feature of this series is the link it provides between the histo-chemical results and the macro-chemical results just described. The former emphasize an accumulation of potassium in meristems and the latter an accumulation in the upper middle regions of stalks.

The present series shows the accumulation in the apex, averaging about 7 per cent. of the dry weight, the dip in the internodes just behind to

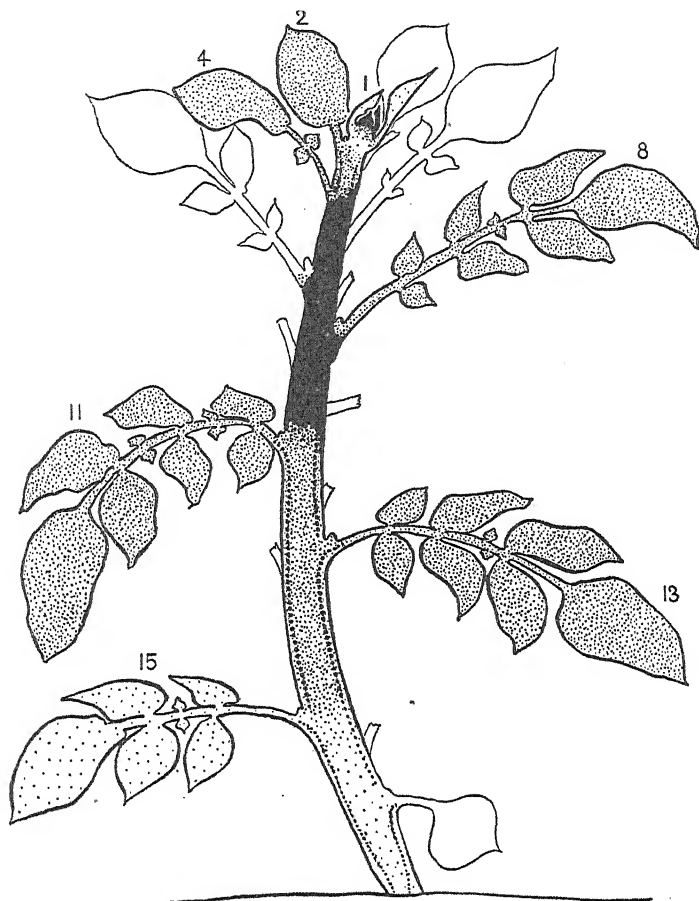


FIG. 5. Diagram of the amount of potassium relative to total dry matter in a potato haulm during active growth. Sparse dotting indicates 0-3 per cent. potassium in the dry weight; closer dotting 3-6 per cent.; full black 6-9 per cent. Only those parts are shaded for which analytical results have been obtained. The remaining leaves are indicated in outline or by the base of the petiole.

about 2.5 per cent., and the beginning of the rise towards the middle of the stem, that eventually attains about 9.5 per cent.

*Third series.* The conditions of sampling made it impossible to obtain the fresh weight, and hence the water content of the plants in the first series. Earlier results (5) showed a close correlation between potassium and water contents at different stages of growth, and also that both concentrations diminished in different organs in the order stems > leaves > tubers. Reference to the literature (Maximov, 8) showed

that the distribution of water in the stems of many plants is similar to the distribution of potassium as shown in the first series (pp. 280-285). It therefore appeared worth while to determine both distributions on the

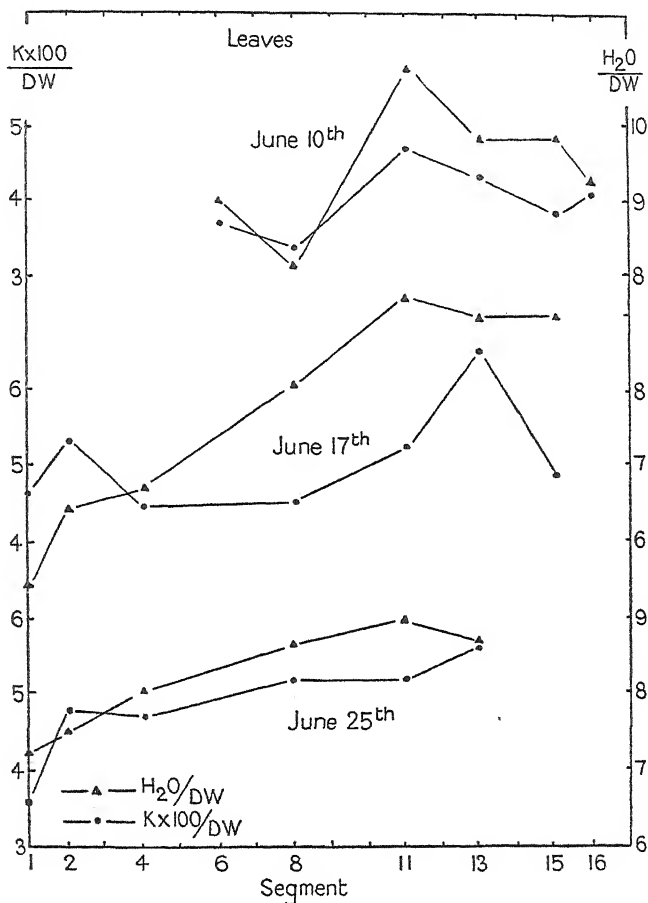


FIG. 6. Distribution between leaves of water and potassium relative to their dry weight, on three occasions of sampling. Growth of the haulms was incomplete on June 10 and the eventual sixth segment was the highest large enough for sampling.

same material, and a third series of samples taken on three separate occasions was obtained for this purpose. The primary data are given in Table VI, and the ratios potassium  $\times 100$ /dry weight and water weight/dry weight are shown in the graphs of Figs. 6 and 7.

The distribution of potassium duly repeats that found in the first series, and the distribution of water follows it closely, whether one is considering the amount per segment or the ratio to the dry matter. Correlation coefficients between the two quantities were calculated for each occasion of

TABLE VI.

*Dry Weight, Water and Potassium in Centigrams per Plant.*

Date.	Segment.	Leaves.			Stems.		
		Dry weight.	Water.	Potassium.	Dry weight.	Water.	Potassium.
June 10	6	4.76	43.2	0.177	—	—	—
	8	6.00	49.0	0.206	1.24	34.8	0.055
	11	12.20	131.8	0.578	4.54	112.4	0.417
	13	11.90	117.1	0.515	6.26	118.7	0.495
	15	6.00	59.2	0.233	5.70	103.0	0.404
	16	1.87	17.3	0.078	4.00	74.2	0.274
June 17	1	4.47	24.3	0.208	0.91	10.6	0.053
	2	8.99	53.5	0.444	2.19	33.5	0.232
	4	16.14	108.2	0.712	4.83	91.3	0.603
	8	22.75	184.3	1.030	10.12	204.9	1.045
	11	19.60	182.0	0.968	15.33	220.6	1.205
	13	9.18	82.5	0.614	9.88	143.4	0.788
	15	1.00	9.0	0.489	7.30	79.3	0.444
June 25	1	17.80	128.9	0.648	3.92	51.4	0.322
	2	22.76	172.3	1.098	9.69	176.7	0.914
	4	24.02	192.7	1.123	10.70	191.0	1.050
	8	22.78	197.2	1.175	11.22	230.5	0.984
	11	16.75	150.9	0.870	15.03	210.0	1.150
	13	11.89	104.8	0.670	18.88	234.5	1.230
	15	—	—	—	9.49	71.71	0.502

sampling and their significance tested by comparison with Fisher's Table 5 A (3). The results obtained were as follows :

TABLE VII.

*Correlation Coefficients (r) for Distribution of Potassium and Water in Potato Haulms.*

## A. Weights per segment.

	r.	Leaves.		r. °	Stems.	
		n.	P.		n.	P.
June 10	0.840	3	0.01	0.995	3	0.01
17	0.927	5	0.01	0.996	5	0.01
25	0.960	4	0.01	0.956	5	0.01

## B. Potassium as percentage of dry weight compared with water-dry weight ratio.

June 10	0.907	4	0.02	0.01	0.823	3	0.10	0.05
17	0.538	5	0.30	0.20	0.913	5	0.01	
25	0.840	4	0.05	0.02	0.850	5	0.02	0.01

*n* = number of degrees of freedom in the comparison.

All correlations in the first section of the table are significant, i.e. the possibility of their arising fortuitously is less than one in twenty ( $P = 0.05$ ). There is, however, a strong correlation with the size of the segment involved

in this case, and this is most simply eliminated by expressing the distributions on the 'dry weight basis', and when this is done the correlations are considerably reduced. Most of them still remain significant, however, and

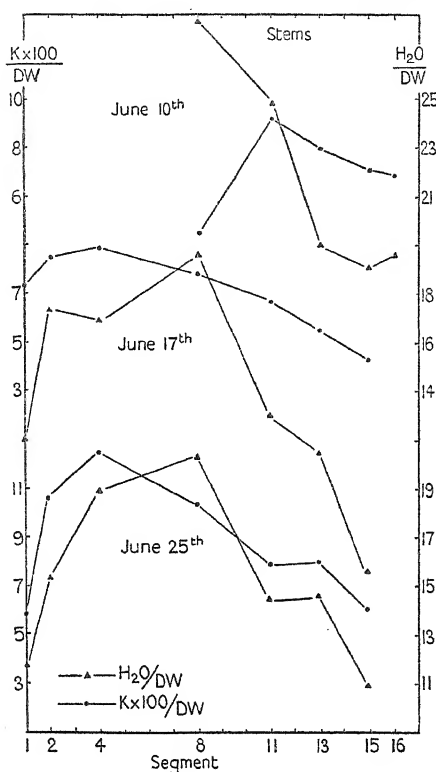


FIG. 7. As Fig. 6 showing the distributions in stems.

the only one which fails by any considerable margin is that for the leaves on June 17th. It is clear that in general the correlation is very strong, i.e. if a single coefficient were calculated for all the data including all the degrees of freedom it would easily reach significance.

#### SUMMARY AND CONCLUSIONS.

The work of the present and two preceding papers enables us to describe the distribution of potassium in the potato plant with confidence. Its chief features are as follows:

1. Potassium is present in all regions of the plant and may compose an exceptionally high proportion of the dry matter: occasional values above 10 per cent. are recorded.
2. Potassium is present in particular abundance in all actively growing

tissues such as stem and root apices, tuber sprouts and reproductive bodies. The average amount of potassium in a given quantity of dry matter is closely related to the rate of dry weight increment. The uptake of further potassium by the plant as a whole is similarly connected. There is thus a close connexion between abundance of potassium and active growth, but there is no evidence that the potassium precedes and provokes the growth. The alternative, that growing tissues have the capacity for collecting the potassium that they may require for further growth is equally allowable, and on the evidence of other lines of work more probable. There is, for example, no probability that potassium is a growth hormone.

3. Proteins being also abundant in meristematic tissues have a rather similar distribution to that of potassium. Both substances appear from histo-chemical studies to be also abundant in sieve-tubes. The older leaves continually lose potassium while at the same time the younger ones gain it and the work of Ruhland and Wetzal with begonia quoted by Onslow (9) suggests a similar movement of amino-acids. The translocation of potassium in the form of salts of amino-acids or proteins seems, therefore, not unlikely.

4. The potato plant 'stores' potassium in the upper middle region of the haulm in considerable quantities. It is not evident that this supply is called upon to any great extent for the formation of tubers, and the dead stalks remain rich in the element.

5. The distribution of water in the stem shows similar features to that of potassium. In the vegetative organs the concentration of potassium relative to water usually varies between 0.5 and 1.0 per cent. It is impossible to suppose that the presence of the water alone controls the accumulation of the potassium, and since proteins are relatively scarce in the middle internodes they cannot be the cause of the accumulation either. The mechanism of this accumulation cannot be deduced merely from observations of its distribution.

6. Results dealing with the distribution of potassium inside the cell must be interpreted with the greatest caution (11). The element appears to be normally present in cytoplasm and vacuole and is plentiful at the surface of nuclei and plastids, though the reagent never succeeds in precipitating it inside them. In view of the marked effect of potassium nutrition on carbohydrate metabolism (4) the presence of the metal at the chloroplast surface is interesting.

7. Histochemical (11) and analytical (5) evidence considered together suggest that a continuous circulation of potassium goes on; movement towards the leaves occurring in the transpiration stream and movement away from them in the phloem. The probable rate of movement (5) is too rapid to result from diffusion alone. Some mechanism such as that suggested by Münch for the translocation of saps seems, therefore, more probable.



It is a pleasure to express our thanks to Dr. A. R. Clapham for his help and advice in the preparation of this paper.

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# Study of the Products of Photosynthesis in Leaves in Artificial and in Natural Light.

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With one Figure in the Text.

## INTRODUCTION.

IN most of the investigations on photosynthesis artificial light has been used as a source of illumination, and the absorption of carbon dioxide or the evolution of oxygen is taken as a measure of the rate of photosynthesis. There has been, however, no evidence that under artificial illumination, the nature and the quantity of products produced on photosynthesis are the same as those produced under natural illumination, nor is it ascertained whether the trend of events after the diffusion of  $\text{CO}_2$  in the assimilating elements is the same or different from that taking place in sunlight. Unless the resulting products under artificial illumination are similar to those produced under natural light, the conclusions drawn from the experiments carried out in artificial light cannot be applied directly to the process as it takes place in nature. Amongst the products of  $\text{CO}_2$  assimilation hexoses, cane-sugar, and starch are generally found in starch-forming leaves. The presence of maltose, though found by early workers, as Brown and Morris (1), is disputed by Davis and Sawyer (6) from observations on the leaves of potato and *tropaeolum*. It is possible that under artificial illumination the process may end at the sugar stage or starch stage or at any other stage, and the conversion of sugar to starch may or may not take place. If this happens to be the case, the data concerning photosynthesis in artificial light may lose much of its value.

The present investigation was undertaken to obtain some comparison of the nature and quantities of the different carbohydrates produced in photosynthetic assimilation under artificial light with those produced under

natural light in the leaves of the same plant with the same period of exposure.

#### INVESTIGATION.

About thirty plants of a species of the same age and of the same previous history as far as possible were kept in a dark-room for thirty-six to forty-eight hours to render the leaves free of starch. Ten of them were exposed in a dark room to the artificial light from a 1,500-watt gas-filled metal filament lamp with a water cooler, 30 in. by 30 in. by 4 in., intervening between the lamp and the plants. The lamp was kept at a distance of 75 cm. from about the middle of the plants. The plants (with pots) were about 2 to 3 ft. in height from the ground. Ten pots could be conveniently arranged beneath the cooler without the overlapping of the leaves. A thermometer was suspended at a distance of 75 cm. from the lamp, with its bulb facing the lamp.

The room was also kept cool by an exhaust fan working throughout the period of experimentation and by keeping a side door of the room open, taking care that no sunlight entered the room from outside. This precaution kept the concentration of carbon dioxide of the room the same as that of the outside air in which similar plants were to be exposed to natural illumination. A thermometer was kept below the cooler, and it showed a very small rise ( $1^{\circ}$  to  $1.5^{\circ}$  C.) in four hours' exposure.

Ten of the plants from the dark room were exposed to artificial light from 11 a.m. to 3 p.m. A second batch of ten plants was exposed to the diffused sunlight for four hours, from 11 a.m. to 3 p.m. The temperature variation during the period of exposure was about  $1^{\circ}$  to  $1.5^{\circ}$  C. The same variation of temperature was maintained in artificial light, by changing the flow of water so that the results obtained might be under identical conditions of temperature in both the cases.

The intensity of light and the distribution of energy in natural light do not remain constant from morning till evening, and so the period of exposure from 11 a.m. to 3 p.m. was purposely selected, since during that period the changes in the intensity of light and in the amount of radiant energy are the least. Whenever plants were exposed in the experiments to the natural light they were placed at the same spot and during the hours mentioned.

The leaves from the third batch of remaining ten plants were immediately taken for extraction of carbohydrates. The method employed was that of Davis, Daish, and Sawyer (7).

#### METHOD FOR ESTIMATING SUGAR ESTIMATION.

Folin and Wu, in estimating the minute quantity of sugar in blood, made use of the fact that when reducing sugars are treated with an alkaline copper sulphate solution, the reduction of the cupric salt takes place and

the cuprous oxide so formed gives blue coloration with phosphomolybdic acid solution, any unreduced copper being at the same time decolorized. This method was later modified by Calvert (2 and 3) and Stanford and Wheatley (8), and it is used here with some further modifications.

Two solutions, A and B, are prepared. (A) alkaline copper sulphate solution. (B) phosphomolybdic acid solution.

Standard Sugar Solution: For the preparation of the standard sugar solution 1 grm. of anhydrous glucose (M.P.  $146^{\circ}\text{C}.$ ) was dissolved in 1 litre of distilled water, and kept in a glass-stoppered bottle with 2 c.c. of toluene in it.

The colorimeter used is the Nephelometer-colorimeter, of the Klett Manufacturing Co., New York. Before using the instrument as a colorimeter the following points were noted: (a) the zero point of the scale and (b) the adjustment of the mirrors. The test-tubes used for the known and unknown sugar solutions should be made of glass of the same specific heat. The sugar solutions obtained from the leaf-extracts in these investigations contain a mixture of sugars. So first, the reducing sugars are estimated in a given solution. Secondly, the cane-sugar is estimated after hydrolysis with citric acid, as recommended by Davis and Daish (5). Thirdly, maltose is estimated after hydrolysis with sulphuric acid.

Starch is hydrolysed to glucose and maltose by taka-diaxase at  $38^{\circ}$ – $40^{\circ}\text{C}.$  So the solution contains a reducing sugar, glucose, and a partially reducing sugar, maltose. Maltose is, therefore, hydrolysed with 10 per cent.  $\text{H}_2\text{SO}_4$  at  $70^{\circ}\text{C}.$ , and then the total reducing sugars thus obtained are estimated by the colorimeter. The value for starch is obtained by multiplying the value of dextrose thus obtained by 0.9.

If the sugar solution contains other substances such as sodium acetate, citrate or sulphate, they have an inhibiting effect on the reducing power of the sugar solution. In other words, the values for sugars obtained would be lower than the true values. The concentration of such impurities as sodium carbonate and sodium acetate are determined and added to the standard solution of sugar.

Plants of *Helianthus annuus* (a small-leaved variety), *Abutilon asiaticum*, *Ricinus communis*, and *Phaseolus vulgaris*, were taken for experiment according to the procedure described above. About thirty plants of each species were kept in the dark for forty-eight hours, and one-third of them were exposed to artificial light for four hours, one-third to natural light for four hours, and the leaves of one-third were taken directly for analysis. The carbohydrates were extracted, purified, and separated in the manner already stated, and estimated colorimetrically. The results are given in Table I. In all experiments the air temperature in the artificial light and the air temperature in the diffused sunlight fluctuated between  $29^{\circ}$  and  $31^{\circ}\text{C}.$  In all cases the results are expressed in grm. per 100 grm. of fresh weight of the leaves.

The results thus obtained gave remarkable differences in the amounts of different carbohydrates. (a) The starch formed in natural light is about fifteen times as great as that formed in the artificial light. (b) The sucrose is formed in a slightly higher quantity in artificial light than that formed in natural light. (c) Total carbohydrates as equivalent to dextrose formed in the artificial light and in natural light are in the ratio of 1:3 approximately. The higher value of total carbohydrates is entirely due to the formation of starch in greater quantity in natural light than in the artificial light.

TABLE I.

(Grm. per 100 grm. fresh weight of leaves.)

	Reducing sugars.	Sucrose.	Starch.	Sugars as hexoses.	Starch as hexoses.	Total carbo- hydrates.
<i>Helianthus annuus</i> L. (small-leaved).						
Dark . . . .	0.1110	0.0341	0.0136	0.1469	0.0151	0.1620
Artificial light .	0.0196	0.1646	0.0432	0.1928	0.0480	0.2408
Diffused sunlight.	0.0219	0.1455	0.4896	0.1750	0.5440	0.7190
<i>Abutilon asiaticum</i> G. Don.						
Dark . . . .	0.0564	0.0416	—	0.1002	—	0.1002
Artificial light .	0.0152	0.3310	0.1521	0.3638	0.1690	0.5328
Diffused sunlight.	0.0215	0.2050	1.6900	0.2368	1.7685	2.0053
<i>Ricinus communis</i> L.						
Dark . . . .	0.0891	0.0339	—	0.1248	—	0.1248
Artificial light .	0.1168	0.0917	0.0699	0.2133	0.0779	0.2911
Diffused sunlight.	0.0407	0.1593	0.4466	0.2085	0.4962	0.7047
<i>Helianthus annuus</i> L. (small-leaved).						
Dark . . . .	0.0017	0.0116	0.0050	0.0139	0.0056	0.0195
Artificial light .	0.0075	0.1069	0.0123	0.1200	0.0137	0.1337
Diffused sunlight.	0.0075	0.1639	0.0265	0.1800	0.0295	0.2095
<i>Phaseolus vulgaris</i> L.						
Dark . . . .	0.0025	0.0135	—	0.0167	—	0.0167
Artificial light .	0.0027	0.0188	0.0180	0.0225	0.0200	0.0425
Diffused sunlight.	0.0030	0.1112	0.0630	0.1200	0.0700	0.1900

When the above experiments were made, the age of the plants and the previous history in any two experiments were not taken into consideration. It was, therefore, decided to make similar experiments with plants of the same age and of similar previous history. Three sets of experiments were made with *H. annuus* (big-leaved), *H. annuus* (small-leaved), *R. communis*, and *A. asiaticum*, and the quantities of carbohydrates formed in the two types of illumination in each of the four species estimated and compared. In order to show clearly the differences between the quantities of sugars,

starch, and total carbohydrates formed in artificial light and in diffused sunlight, the ratios of the substances formed in the two lights are also given (Table II).

In the experiments described below, the intensity of the artificial light as measured by a thermopile, was higher than that of the sunlight, as will be shown later. In the results with *A. asiaticum* (Table III) the total intensity of the artificial light was made equal to that of the sunlight as measured by a thermopile. The differences in the carbohydrates formed in the two illuminations still remained.

TABLE II.

<i>Helianthus annuus</i> (small-leaved variety).			
	Set I.	Set II.	Set III.
Total sugars as hexoses.			
Artificial light .	0.0180	0.0177	0.0180
Diffused sunlight	0.0193	0.0180	0.0178
	1:1.0	1:1.0	1:1.0
Starch as hexoses.			
Artificial light .	0.0115	0.0101	0.0150
Diffused sunlight	0.0258	0.0342	0.0302
	1:2.3	1:3.3	1:2.0
Total carbohydrates as hexoses.			
Artificial light .	0.0295	0.0278	0.0330
Diffused sunlight	0.0451	0.0522	0.0480
	1:1.5	1:1.8	1:1.5
<i>Helianthus annuus</i> (large-leaved variety).			
Total sugars as hexoses.			
Artificial light .	0.0218	0.0165	0.0135
Diffused sunlight	0.0146	0.0119	0.0096
	1.5:1	1.4:1	1.4:1
Starch as hexoses.			
Artificial light .	0.0158	0.0165	0.0172
Diffused sunlight	0.0477	0.0430	0.0458
	1:3	1:2.6	1:2.7
Total carbohydrates as hexoses.			
Artificial light .	0.0376	0.0330	0.0307
Diffused sunlight	0.0623	0.0549	0.0554
	1:1.7	1:1.5	1:1.8
<i>Ricinus communis</i> .			
Total sugars as hexoses.			
Artificial light .	0.0500	0.0400	0.0694
Diffused sunlight	0.1050	0.0480	0.1111
	1:2	1:1.2	1:1.6
Starch as hexoses.			
Artificial light .	0.0152	0.0769	0.0360
Diffused sunlight	0.0276	0.1690	0.0847
	1:1.8	1:1	1:2.3
Total carbohydrates as hexoses.			
Artificial light .	0.0652	0.1169	0.1054
Diffused sunlight	0.1326	0.2170	0.1958
	1:2	1:1.8	1:1.8

The study of the ratios of total sugars, starch, and total carbohydrates under two types of illumination shows clearly the differences in the amounts formed. The ratios show fair agreement in the three sets of determinations made with each plant. The carbohydrates produced in diffuse sunlight are nearly twice those produced in artificial light.

It is seen that the higher ratio of the total carbohydrates formed in diffuse daylight is due to the higher production of starch in the sunlight than in the artificial light. It may be argued that the artificial light is poor in certain radiations which are responsible for the conversion of sugars into starch, and therefore the photosynthetic machinery is clogged by the accumulation of sugars in the artificial light.

TABLE III.

*The Total Radiation from the Two Sources of Light was made the same.*

<i>Abutilon asiaticum.</i>			
	Set I.	Set II.	Set III.
Total sugars as hexoses.			
Artificial light .	0.0265	0.0200	0.0158
Diffused sunlight	0.0375	0.0278	0.0367
	1:1.4	1:1.4	1:2.2
Starch as hexoses.			
Artificial light .	0.0240	0.0280	0.0388
Diffused sunlight	0.0941	0.1022	0.1048
	1:3.9	1:3.4	1:2.7
Total carbohydrates as hexoses.			
Artificial light .	0.0496	0.0480	0.0546
Diffused sunlight	0.1316	0.1300	0.1415
	1:2.6	1:2.7	1:2.6

If the above reasoning is correct, there should be no increase in the production of carbohydrates in sunlight in comparison to those formed in artificial light in leaves which normally do not produce starch.

To test the above view, a series of experiments was performed with the leaves of the onion plant which does not produce starch in photosynthesis. Table IV gives the results of estimations of carbohydrates formed under artificial and natural illuminations.

The total amounts of sugars formed in the artificial light in the three sets of experiments are nearly the same. They are 94.5, 97.4, and 92.4 mg. per 100 grm. of fresh leaves. The ratios of carbohydrates formed under artificial and natural illuminations are 1:3, 1:4, and 1:3.4 in the three sets.

The results clearly indicate that the differences between the amounts of photosynthetic products in artificial light and in natural light are not caused by the non-formation of starch, as the total sugar produced in sunlight is greater than that produced in the artificial light.



Two kinds of differences are expected in the lights obtained from these two sources. (a) The total intensities of the lights that fall upon the leaves may be different in the two cases; (b) and secondly, the quality of the light may be different.

TABLE IV.  
*Allium cepa* L.

Date	29. 8. 1929.	13. 9. 1929.	19. 9. 1929.
	Set I.	Set II.	Set III.
	Total reducing sugars.	Total reducing sugars.	Total reducing sugars.
Dark . . . .	0.0113	0.0359	0.2076
Artificial light . .	0.1958	0.1333	0.3000
Diffused sunlight .	0.3000	0.4200	0.5200

The results obtained with *A. asiaticum*, when the total intensity of the artificial light was made equal to that of the diffused sunlight, do not indicate that the differences in the carbohydrates formed in two lights are due to the differences in their total intensity.

The measurements of the light intensity were made with a micro-thermopile, and it was found, as a result of several experiments, that the total radiation from the artificial light from the electric lamp was nearly double that of the diffused light. With panchromatic photographic plates as a basis of comparison, the intensity of the diffused sunlight was nearly double that of the artificial light. The qualities of the light from the two sources is markedly different, as is well known.

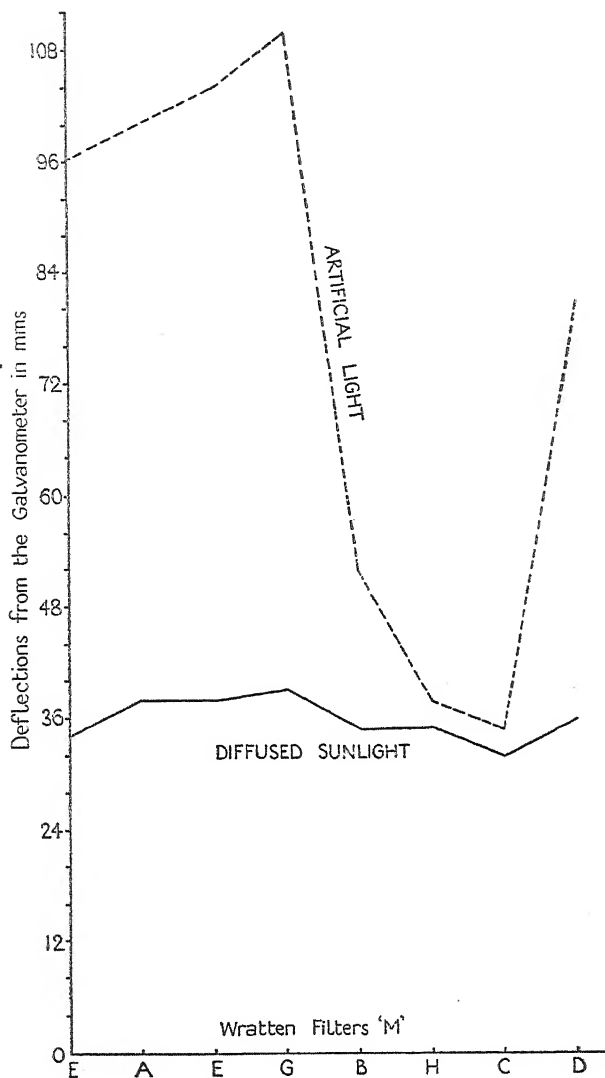
A rough determination of the intensity of different parts of the spectrum of the two lights was made by the use of glass Wratten 'M' filters manufactured by the Kodak Co. The intensity of the different spectral regions from the two sources of light of equal total radiation were measured by a micro-thermopile and by the photographic plate. The results are given on p. 302 (Table V). A graph showing the intensities of the different radiations in the two lights as measured by the micro-thermopile is given on p. 303.

It is seen that the red rays from the two sources have equal effect on the photographic plate, while the green radiations from the artificial light are slightly stronger than those from sunlight. The sunlight on a photographic basis is about three to four times as strong as the artificial light in the blue and the blue violet regions.

In the present state of our knowledge no further deduction is possible than that the differences in the production of carbohydrate material in the two types of illumination are due to differences in the quality of the radiation received.

TABLE V.

Visual colour.	Filter.	Spectral transmission.	Thermopile		Photographic plate.
			Galvanometric deflection (cm.). Diffuse light.	Galvanometric deflection (cm.). Artificial light.	
Pure red .	F	6100 to red end	3.4	9.6	Artificial light = natural light.
Orange red .	A	5800 to red end	3.8	10.0	
Orange .	E	5600 to red end	3.8	10.4	
Strong yellow	G	5100 to red end	3.9	11.0	
Green .	B	4600 to 6000	3.5	5.2	Artificial light is stronger than natural light.
Blue .	H	4200 to 5400	3.5	3.8	Natural light much stronger than artificial light.
Blue violet .	C	4000 to 5100	3.2	3.5	Natural light much stronger than artificial light.
Violet . .	D	$\left. \begin{array}{l} 3800 \text{ to } 4600 \\ \text{and} \\ 6400 \text{ to red end} \end{array} \right\}$	3.6	8.1	Natural light much stronger than artificial light.



## SUMMARY.

The nature and quantities of the different carbohydrates formed in leaves exposed to artificial light (gas-filled electric lamp over a water screen) and to diffuse sunlight, and under uniform and comparable conditions of experimentation, were determined. A colorimetric method was used for sugar determination.

The results obtained for different species indicate that—(a) with starch-forming leaves the starch formed in artificial light is only about one-third

of the amount formed in the natural light. (b) The sucrose formed under both conditions is approximately equal. (c) The total carbohydrates formed under artificial light are less than one-half of those formed in the diffused sunlight.

That the increased formation of carbohydrates in the diffused sunlight is not due to the increased formation of starch can be seen from the results obtained with the leaves of onion (*Allium cepa*), which does not produce starch. In the leaves of this plant the total amount of sugar in the diffuse sunlight is over three times that in artificial light.

The differences in the amounts of total carbohydrates are not due to the differences in the total intensity of the two lights, but are due to the differences in the quality of the radiation.

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# Studies in the Physiology of the Appressorium of *Colletotrichum gloeosporioides*.

BY

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With Plate XII.

## CONTENTS.

	PAGE
I. INTRODUCTION . . . . .	305
II. DESCRIPTION OF THE FUNGUS . . . . .	306
III. FORMATION OF THE APPRESSORIUM . . . . .	307
IV. GERMINATION OF THE APPRESSORIUM . . . . .	308
V. INFECTION OF THE HOST . . . . .	309
VI. SUMMARY . . . . .	311
LITERATURE CITED . . . . .	312
EXPLANATION OF PLATE . . . . .	312

## I. INTRODUCTION.

THE fungi belonging to the class of *Colletotrichum* and *Gloeosporium* have long been known to produce the peculiar adhesion organs when their germ-tubes come in contact with a hard surface. Hasselbring (10) proved conclusively that in *Gloeosporium fructigenum* these adhesion organs or appressoria (7), formed as a result of stimuli from mechanical contact acting on the germ-tubes, served to attach the fungus to the surface of its host during the early stages of infection. The writer (4) studied the earlier stages of infection of the bean pod by *Colletotrichum Lindenmuthianum*, and showed that a peg-like 'infection hypha' grew out from the surface of the appressorium in contact with the host, which penetrated the cuticle by mechanical pressure. The mechanism of penetration of this fungus was similar in every respect to that of *Botrytis cinerea* (1).

No attempt was, however, made in these studies to find out the exact nature of the stimulus which caused the appressorium to send out the infection hypha. It appeared to Brown (2) that most likely the stimulus

to penetration in such cases was a contact one, although he hinted that chemical factors might influence the nature and degree of this 'thigmotropic' response.

In the present work an attempt is made to find out the nature of the stimulus which induces the fungus, *C. gloeosporioides*, isolated from the anthracnose spots on the leaves of *Citrus medica*, var. *acida*, to cause infection, and to find whether in its method of penetration into the host tissue it agrees with that of *C. Lindemuthianum*. It also records the observations on the morphology of the appressorium of the fungus.

## II. DESCRIPTION OF THE FUNGUS.

The fungus was isolated from the leaf spots of *C. medica* (lemon). The spotted leaves were washed with a jet of water and dipped in 0.1 mercuric chloride solution for 30 minutes, after which they were washed repeatedly with sterilized water. Small pieces containing the spots were then cut out with sterilized scissors, quickly washed with absolute alcohol and flamed to remove traces of the alcohol. These were dropped in Petri-dishes containing 2.5 per cent. rice-agar medium. In the majority of the dishes there was a copious growth of fungal hyphae within 36 hours at room temperature (80° F). Hyphae from the extreme edge of the growth were then transferred to autoclaved leaves of lemon kept in a moist chamber. Boiled lemon leaves were preferred to other media, as from it the fungus was likely to obtain its natural nutriment, and also because the spores produced on them were easier to handle without any chance of the medium itself being transferred with the spores. Within three days of inoculation of the boiled leaves, there was abundant growth of white loose aerial mycelium. The portion of the leaf surface which was not obscured by the white mycelial growth soon became dotted over with salmon-coloured globular masses of spores. As the cultures aged, black dots (stromata) appeared thickly interspersed with the spore masses. They could also be seen developing below some of the latter. The majority of them were smaller than these spore masses and were covered with a white mycelial growth. The larger ones were crowned with the salmon-coloured mass of spores, some having more than one crown. Most of the spores adhering to the stroma could easily be removed by washing in water. The mature stroma then appeared as a more or less prominently raised dark compact body having one or more shallow depressions on the surface. The spores collected in masses in these depressions and appeared as globular crowns. The stroma consisted of a compact mass of knotted or beaded hyphae from which numerous short conidiophores arose, each abstricting off from its tip a number of spores in succession. Some of the short branches, instead of bearing conidia developed into stiff-pointed bristles or

setae with four or five septa, the older ones becoming dark in colour. The spores were hyaline, nonseptate, rounded at the ends, slightly constricted in the middle, with two prominent refractile globules at two ends. They measured on an average  $14.7\ \mu$  long and  $4.9\ \mu$  broad. The size of the conidia, however, was not a constant character (3). It varied according to the nature of the medium in which the fungus was growing. While the spores collected from the dry twigs under natural conditions measured  $12.2\ \mu \times 4.3\ \mu$ , those produced in 2.5 per cent. dextrose-agar medium were  $11.2\ \mu \times 5.3\ \mu$ . The latter contained a number of globules instead of only two as in the other cases.

### III. FORMATION OF THE APPRESSORIUM.

Hanging-drop preparations were made of a suspension in sterile tap-water of the spores from a three days old culture on boiled lemon leaves. They were kept in the laboratory where the maximum temperature during the day rose to  $80^{\circ}\text{F}$ . The majority of the spores in these preparations remained floating in the convex drops of water. Within ten hours most of the spores germinated. There was a strong indication that germination of the spores was closely related to the temperature conditions. At  $85^{\circ}\text{F}$ . it was poor, while at  $90^{\circ}$ – $95^{\circ}\text{F}$ . there was hardly any germination. Edgerton (5) has established this relationship in the case of a number of species of *Gloeosporium*, but it has not been thoroughly worked out with *C. gloeosporioides*.

Before germination a septum appeared in the middle of the spore, dividing it into two cells, each containing a prominent refractive globule. These globules seemed to be of oily nature as they stained dark brown with osmic acid. A germ-tube was put out usually from both the cells of the spore. During this process the droplets broke up into smaller ones, which disappeared with the further growth of the germ-tubes. The tips of the germ-tubes developing from the spores sticking to the surface of the cover-glass soon came in contact with the hard glass surface, and instead of elongating, they developed into appressoria. But those from the floating spores grew into long filaments with branches at frequent intervals. Most of these branches, after about 24 hours growth, began to abstrict conidia from their ends. Some of the branches which grew up towards the cover-glass soon came in contact with its hard surface, their growth in length was arrested, and their tips developed into appressoria. New branches growing behind these appressoria started producing conidia from their loose ends. Some of the free branches which were actively producing conidia were induced to develop appressoria instead by making them touch the glass surface. This was done in the following manner. A few fine stiff bristles were passed through the vaseline used for cementing the cover-glass in the

hanging-drop preparation, thereby establishing communication of the drop with the outside air. The hanging drop gradually contracted through evaporation and forced the ends of some of the hyphae formerly producing conidia to come in contact with the surface of the cover-glass. The bristles were then removed and the cover-glass pressed down again. All these branches developed appressoria at their tips (Pl. XII, Figs. 3, 5*a*, 5*b*, 5*c*, 5*d*). The formation of appressorium at the end of a hypha growing in water, was therefore due to the mechanical stimulus resulting from its contact with a hard surface which obstructed its apical growth. Under similar stimulus appressoria were induced even on those hyphae which were actively producing conidia before.

Pl. XII, Figs. 3-4, 4*a*, 4*b*, 4*c*, 4*d* show the process of formation of an appressorium on one of the branches. The tip of the hypha in contact with the hard surface of the glass was obstructed in its process of elongation, and very soon swelled into a small spherical vesicle. A number of oil drops appeared therein, and a little distance behind it a partition wall was formed. The walls of the vesicle, thus cut off from the hypha, slightly thickened and assumed a dark brown colour. While these changes occurred in the walls, the small droplets within the vesicle coalesced into one large prominent shiny globule. These globules stained dark brown with osmic acid and were thus of oily nature, similar to the globules in the conidia noted above. They are very likely the reserve food material for the later development of the appressoria. Hasselbring (11) probably mistook similar globules in the appressoria of *G. fructigenum* for germ-pores.

The shape of the appressorium depended on its angle of contact. When the apex of the hypha touched the glass surface slantingly, as was usually the case, the appressorium was a pear or flask-shaped structure. When it touched the glass surface perpendicularly its shape was irregular and globose. The margin of the hyphal tube in the latter case appeared as a minute circular ring in the middle of the appressorium (Pl. XII, Figs. 5*c*, 6*b*).

#### IV. GERMINATION OF THE APPRESSORIUM.

In pure water the appressoria of *C. gloeosporioides* remained dormant indefinitely without showing any sign of germination. On the other hand, when a drop of dilute glucose solution (0.1 per cent.) was added to the hanging drop the appressoria became active within a very short time. They did not appear to have any 'rest period'. The refractile globule within the appressorium broke up into a number of smaller ones, and within three hours, at 80° F., a germ-tube protruded from its wall, which gradually elongated into a hypha. With further growth of this germ-tube the globules disappeared and the appressorium was left empty. The germination of the appressorium consisted, like that of the spores, in a



protrusion of its wall at any point and elongation of the swelling into a hypha. When the germ-tube protruded from a point directly opposite its adpressed surface, its end could be seen as a circular ring on the wall of the appressorium. No germ-tubes were seen growing out from the side of the appressorium which was closely pressed on the glass surface.

The appressoria germinated in a similar way in the juices of prunes, turnip, and orange.

Hasselbring (11) found that the appressoria of *G. fructigenum* could germinate in sugar-beet infusion even after prolonged drying. But the appressoria of *C. gloeosporioides* behaved differently in this respect. When the hanging drop containing them was allowed to dry up the appressoria immediately shrank and collapsed, their walls being thrown into folds (Pl. XII, Figs. 7a, 7b, 7c). They failed to recover from this condition either in water or in nutrient solution even after three days. The appressoria of this fungus are thus unable to withstand drying, and it is doubtful whether they can be considered as resistant organs of propagation.

## V. INFECTION OF THE HOST.

Infection of the young leaves of the lemon could easily be brought about by sowing the spores, suspended in tap water, on their surface. Fawcett and Lee (7) stated that *C. gloeosporioides* usually attacked only the mature leaves, but they did not describe the manner in which this was done. In spite of repeated trials the writer failed to induce infection of the mature leaves by sowing spores on them. Very young leaves about 1 cm. long and 0.5 cm. broad borne on young succulent twigs were therefore used. The cut ends of these twigs were kept dipped in water in small beakers. The spores from a four days old culture on a boiled lemon leaf were stirred thoroughly in a little sterile water until a faintly milky appearance was reached. Small drops of this suspension were placed on the surface of the leaf which was previously washed thoroughly with sterile water to remove dust and foreign spores. The surface of the leaf being smooth the drops could not be spread out into thin films to give uniform condition of oxygen supply. The drops were accordingly made as small as could be done with a 2 mm. platinum loop. Moist chambers containing these inoculated leaves were kept in the laboratory at a temperature of 80° F.

Within 12 hours of sowing a large number of appressoria were visible under the low power of the microscope throughout the area under the infection drop, being more numerous in the depressions along the small veins where the spores were seen collected in larger number. After 72 hours minute brown spots could be detected. Unlike bean pods (4), such spots were not observed where drops of pure water were used instead of the spore suspension.

The portions of the leaf below the 'infection drops' after 20 hours were fixed in Carnoy's fluid at different intervals. Sections  $6\ \mu$  thick were cut and stained with Heidenhain's iron-alum haematoxylin and counter-stained with orange G in clove oil.

In its method of penetration this species agreed with that of *C. Lindemuthianum* (4). A slight depression appeared in the cuticle below the appressorium due to the pressure exerted by the latter (Pl. XII, Fig. 8). A very narrow infection hypha protruded from the appressorium from its adpressed surface, which pierced through the thin cuticle and then caused dissolution of the subcuticular layers (Pl. XII, Fig. 9). This disorganization of the cellulose layers below the cuticle could be seen as a clearer area round the 'infection hypha'. There was no evidence of disorganization of the cuticle previous to the entry of the infection hypha. Like the infection of the bean pod by *C. Lindemuthianum* (4), the entry of *C. gloeosporioides* into the citrus leaf was effected by a puncture of the turgid cuticle as a result of the mechanical pressure exerted by the 'infection hypha'.

Once the fungus gained entrance through the cuticle its hyphae spread rapidly through the host cells bringing about their collapse and death. The brown spots seen below the infection drop after 72 hours, which were noted above, marked the places where the fungus had advanced sufficiently in the leaf tissue to cause death of the underlying cells.

It has been stated above that germination of the appressorium takes place only in the presence of nutrient substances. Brown (2) has shown that substances escape in amount sufficient for the germination of fungal spores from uninjured petals of *Cereus*, *Phyllocactus*, and certain varieties of tulip, into drops of distilled water placed on them. Nutrient substances may similarly diffuse out through the cuticle of the lemon leaves and supply the required stimulus for the development from the appressoria of the infection hyphae. This question was investigated by the following method. Small droplets of doubly distilled water were placed on the uninjured surface of young leaves of lemon. They were re-collected after varying lengths of time by means of fine pipettes, and their power of inducing germination of the appressoria was tested by applying them to the hanging-drop cultures containing ungerminated appressoria. Very young leaves, light green in colour, measuring 1.5 to 2 cm. long and about 0.5 cm. wide were used, as in the investigation of the process of infection. These leaves were previously washed repeatedly in distilled water. Droplets of doubly distilled water were placed on them, and they were then kept in a sterilized moist chamber to prevent the latter from drying up. No browning or any other sign of injury to the epidermis below these droplets due to osmotic disturbance was noticeable when the latter were collected.

Before these droplets were applied to the hanging-drop cultures, the

latter were repeatedly washed with drops of distilled water, removing the excess water each time with a fine capillary tube. This was done in order to ensure that the 'staling principles' (13), which might have been excreted by the fungus and accumulated in these cultures, should not inhibit germination of the appressoria. The appressoria were adhering firmly to the glass surface and were not disturbed by this washing.

It was observed that a good number of the appressoria germinated in the droplets collected from the leaf surface after 72 hours or more. The germ-tubes, however, were very narrow, unlike the thick germ-tubes (Pl. XII, Fig. 2) produced in richer nutrients.

There was thus an indication that the infection hypha grew out of the appressoria adhering on the leaf surface as a result of the stimulus derived by them from the substances diffusing out through the thin cuticle.

Similar experiments were carried out with old mature leaves. But the droplets collected therefrom were unable to effect germination of the appressoria. This may be explained by the fact that the cuticle in the older leaves is thicker and therefore the nutrient substances cannot escape through it to supply the necessary stimulus to the appressoria. The inability of the appressoria to germinate is thus one of the reasons why there was no infection in the old leaves from the spores sown on them.

#### SUMMARY.

The fungus *Colletotrichum gloeosporioides* Penz, isolated from the anthracnose spots on the leaves of *Citrus medica acida* is described.

The spores germinate readily in pure water. The tip of the germ-tube coming in contact with a hard surface swells into a vesicle which gradually develops into a dark-coloured appressorium. This appressorium may develop at the tips of older hyphae which before were actively producing conidia. The process of development of the appressorium is described.

The appressoria of *C. gloeosporioides* are unable to withstand drying.

The germination of the appressorium takes place only in presence of nutrient substances.

There is an indication that substances capable of stimulating germination of the appressorium diffuse out through uninjured cuticle of young leaves into drops of water placed on them.

Stimulated by the substances diffusing out through the cuticle, the appressorium sends out a fine infection hypha from its adpressed surface, which by a continuous and increasing pressure on the cuticle finally ruptures it mechanically. In the mechanism of penetration into the host tissue *C. gloeosporioides* agrees with that of *C. Lindemuthianum*.

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## EXPLANATION OF PLATE XII.

Illustrating Mr. P. K. Dey's paper on 'Studies in the Physiology of the Appressorium of *Colletotrichum gloeosporioides*'.

All figures were drawn with the camera lucida from hanging-drop preparations except Figs. 8 and 9. The host tissue in Figs. 8 and 9 is that of the leaf of *Citrus medica*.

Fig. 1. Germinating spore.  $\times 700$ .

Fig. 2. The ends of hyphal branches after eight hours in 1 per cent. glucose solution. They are much thicker than the original hypha which was growing in tap water, and are filled with coarse granules.  $\times 700$ .

Fig. 3. Appressoria developed at the tips of the branches which have come in contact with the cover-glass. The addressed surface is clearly seen in some of the appressoria. The tip marked A is about to develop an appressorium; drawn at 7.30 a.m.  $\times 700$ .

Fig. 4 (a) The swollen tip of the hypha in Fig. 3 A, drawn at 8 a.m.  $\times 700$ . (b) The same drawn at 9 a.m. A wall has appeared behind the vesicle. The droplets are more prominent.  $\times 700$  (c) The same drawn at 10 a.m. The walls of the appressorium have slightly thickened. The droplets are gradually coalescing into one.  $\times 700$ . (d) The same drawn at 4 p.m. The appressorium is complete with thick walls and a single refractive droplet. The addressed surface is quite clear.  $\times 700$ .

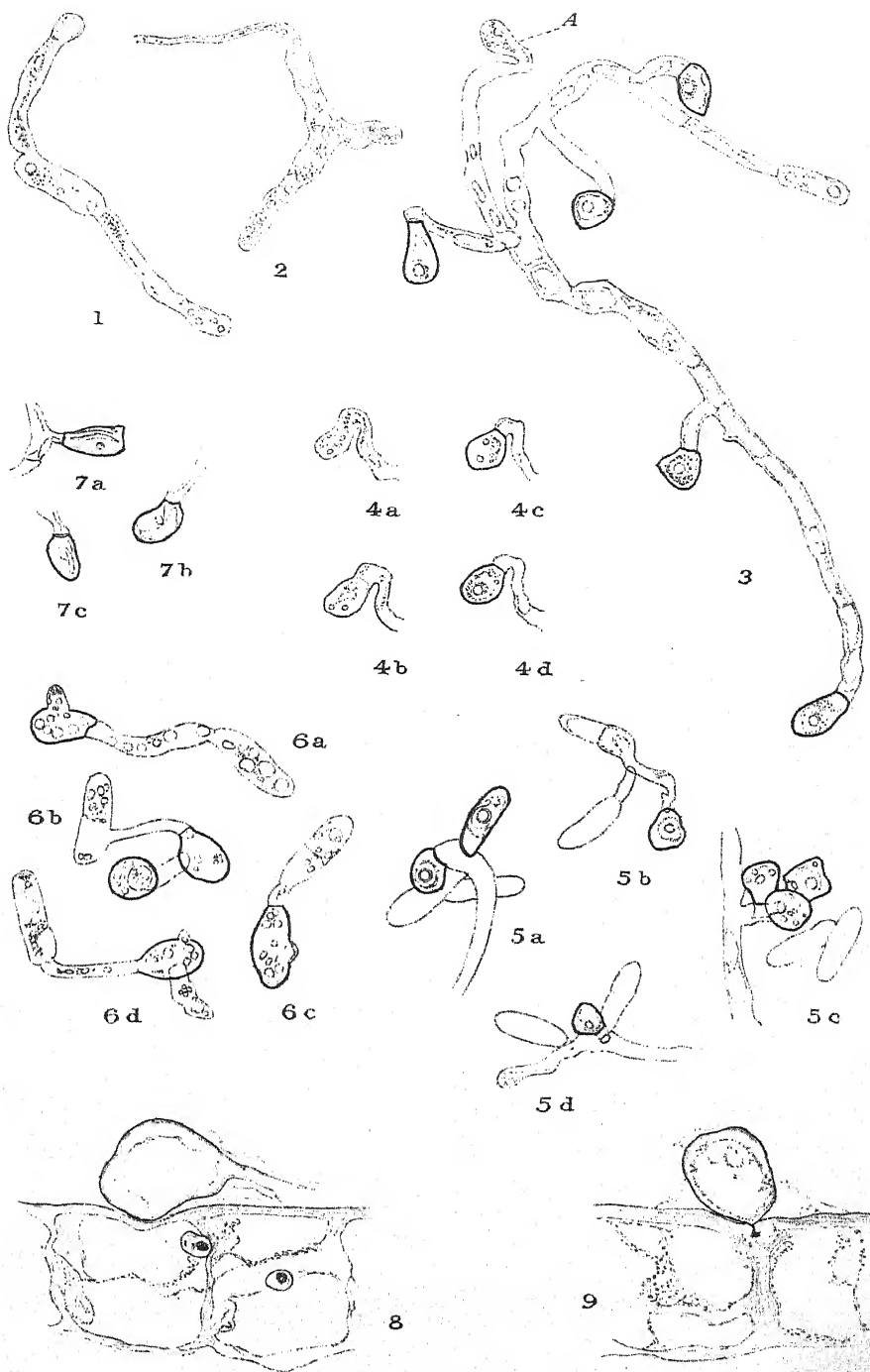
Fig. 5 (a)-(d). Some of the hyphal branches which were abstricting conidia before have been made to touch the glass surface. Their tips have now developed into appressoria. The end of the hyphal tube is visible in the middle of some of the appressoria.  $\times 700$ .

Fig. 6 (a)-(d). The appressoria germinating in 1 per cent. glucose solution. In place of one big refractile droplet a large number of smaller ones have appeared.  $\times 700$ .

Fig. 7 (a)-(c). The appressoria have collapsed and their walls are thrown into folds as a result of the hanging drop containing them being allowed to dry up.  $\times 700$ .

Fig. 8. An appressorium attached to the epidermis of the lemon leaf. A slight protuberance on the appressorium has caused an indentation of the wall of the epidermis.  $\times 1500$ .

Fig. 9. An infection hypha has grown out from the appressorium and effected its entrance into the subcuticular layer by perforating the cuticle. Disorganization of the cell-wall is clearly seen. The tip of the infection hypha has swollen into a vesicle.  $\times 1500$ .





# The Roots and Habit of *Heterangium Grievii*.

BY

M. BENSON.

With Plate XIII and three Figures in the Text.

FROM a block of the famous plant-bearing rock of Pettycur, Fifeshire, Scotland, given me by the late Professor Bayley Balfour in 1905, a series of transverse sections through a single stem of *Heterangium Grievii* exposed on its surface, revealed roots in continuity with the stele and branching in the cortex. As these roots often ran parallel with the stem stele they were also cut in the transverse plane and supplied material for comparison with other roots found accompanying the stems of *Heterangium*. We thus secured evidence of the structure of the roots of this plant which had been previously unknown (Pl. XIII, Figs. 1 and 2).

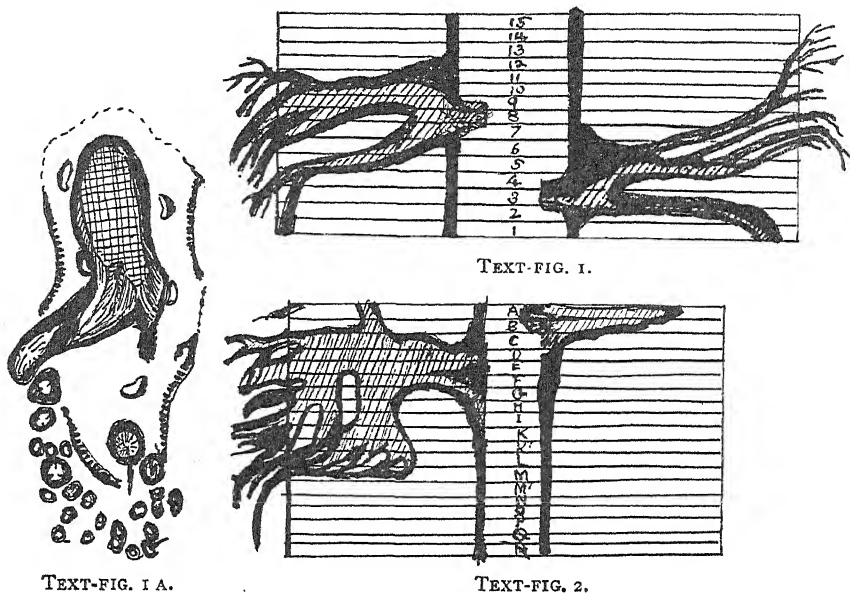
Dr. Scott recorded these observations in his 'Studies in Fossil Botany', vol. ii, ed. 2, p. 411; cf. vol. ii, ed. 3, p. 11. He now asks me to put the evidence on record in detail. Meanwhile further evidence has been secured by myself (R.H.C., C.N., 269, 1-16), Professor Gordon, and Dr. Scott. The original series of sections (R.H.C., C.N., 183, A-R) is retained in the Botanical Laboratory of the Royal Holloway College, Englefield Green, Surrey.

## *Structure of roots and relation to stem.*

As in *H. Lomaxii* and *Lyginopteris* of the Coal Measures, the roots of *H. Grievii* are adventitious and of endogenous origin and branch monopodially (Pl. XIII, Figs. 3, 4, and 5). The radicleferous stem stele is generally elliptical in section and the roots are emitted from the ends of the major axis of the ellipse or from one side of the stele. They branch repeatedly in the cortex, and often send their branches through the cortex a considerable distance in opposite directions parallel to the stem stele (Text-figs. 1 and 2). Sometimes they travel in a leaf-base, and may be crowded as many as ten together in a solid mass. Ultimately on emerging they give rise to a nest of small roots just outside the cortex, where I have counted as many as five and twenty in one section.

The parent roots on leaving the stem stele are large. That cut in

183, C-H, must have been over half a centimetre in diameter, and the curious cluster of relatively large branches in the cortex of Scott, Private Collection, No. 405, almost suggests a water storage organ (Pl. XIII, Fig. 6).



TEXT-FIG. 1. A diagram made by plotting the planes of section of the roots in the slides labelled R. H. C., C. N., 269, 1-15. It demonstrates that the roots may grow up or downwards.

TEXT-FIG. 1 A. A map of the stem in slide R. H. C., C. N., 269, 8. It indicates the close proximity in this case of the points of emission of the two roots shown in Text-fig. 1 and the crowded mass of rootlets just outside the stem.

TEXT-FIG. 2. A diagram made by plotting the planes of section of the roots in the slides R. H. C., C. N., 183 A-R. That on the right of the figure was emitted at right angles to that on the left. Cp. Pl. XIII, Figs. 3 and 4.

Where the root leaves the stem stele the primary wood is connected along a line of two or three millimetres in length with a stem primary bundle. The surrounding region of the stem xylem is locally thickened by secondary elements which are segmented. Each segment is attached to a secondary tracheide, and these are disposed longitudinally in radial plates around the primary core of the root. These plates alternate with parenchyma rays so that in transverse section, if the parenchyma has perished, the root has a spider-like appearance (Pl. XIII, Fig. 2). The pericycle and phloem of the stele are continuous with that of the root, and are often of considerable thickness. The primary roots are probably triarch, but the branches may be diarch or triarch (Pl. XIII, Fig. 5). Many are difficult to discriminate from polyarch roots, so insignificant in amount may be the protoxylem. The secondary phloem is occasionally very fairly preserved.



*Habit of H. Grievii.*

None of the cases met with support the view that the adventitious roots were aggregated to a basal part of the stem. The lateral occurrence of the roots on a stem of elliptical section when radiciferous, the undisturbed phyllotaxy and the large size of the leaf bases occupied by these roots, point to fixation and absorption from a lateral source of water and nutriment.

Moreover, the independence of the stimulus of gravitation shown in the opposite direction of the routes taken by the branches points clearly to their response to a chemical stimulus. It is interesting to note from the diagrams (Text-figs. 1 and 1 A) when compared together that the area of absorption from the two roots was concentrated in a disc-like region. It is about such a region that a quantity of amorphous and decayed matter and coprolites is to be seen in many slides.

*H. Grievii* would seem to have lived among moist rocks and boulders, in the crevices of which, and against their upright surfaces, it could obtain an ample supply of water and humus—thus showing in its habit another feature comparable with the large Hymenophyllaceae so common in New Zealand at the present day.

## EXPLANATION OF PLATE XIII.

Illustrating Dr. M. Benson's paper on 'The Roots and Habit of *Heterangium Grievii*'.

Fig. 1. Part of the transverse section of a radiciferous stem of *Heterangium Grievii* showing a branch root in transverse section. Cp. Text-fig. 2. R. H. C., C. N., 183 K.

Fig. 2. The same branch root as occurs in Fig. 1, but more highly magnified to show the characteristic appearance of the diarch type in more detail. R. H. C., C. N., 183 K.

Fig. 3. A transverse section of the same stem at level of emission of a root on the flat side of the ellipse. Cp. Text-fig. 2 where the root is shown on the right. In Fig. 3, one sees to the right some indication of the envelope of the other root which is seen in median plane in Fig. 4. R. H. C., C. N., 183 A.

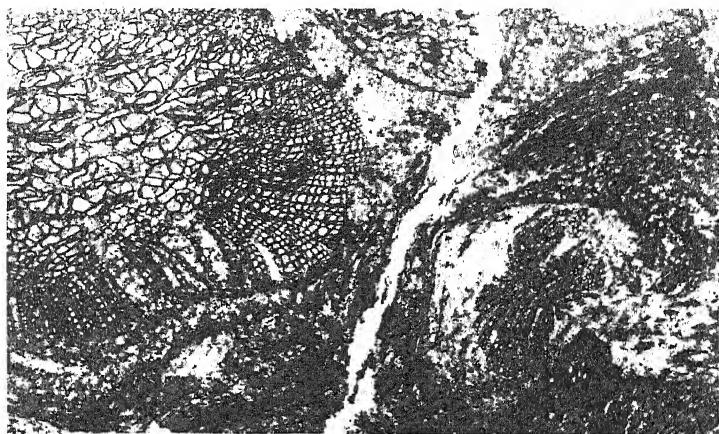
Fig. 4. An almost median longitudinal section of a root leaving at right angles to that in Fig. 3. The protoxylem elements of the stem with which it was probably in continuity can be seen at P, and directly beneath can be seen the secondary xylem elements of the root as they attach themselves to the segmented secondary tracheides locally formed on the stem stele. R. H. C., C. N., 183 E.

Fig. 5. A triarch rootlet showing monopodial branching.

Scott, Private Collection, no. 408.

Fig. 6. A remarkable group of root branches in the cortex of *Heterangium*. The slide was made on the Walton film method by Mr. Hemingway. Scott, Private Collection, no. 405.

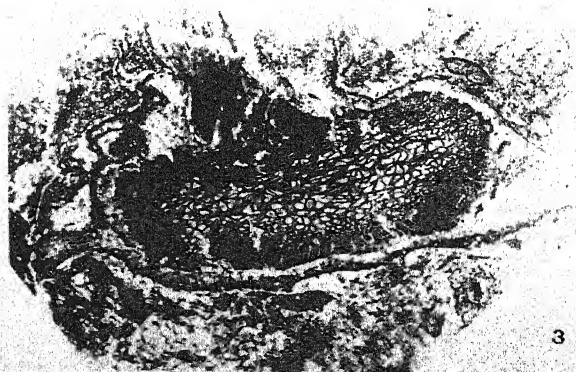
The six micrographs were taken by Mr. Tams.



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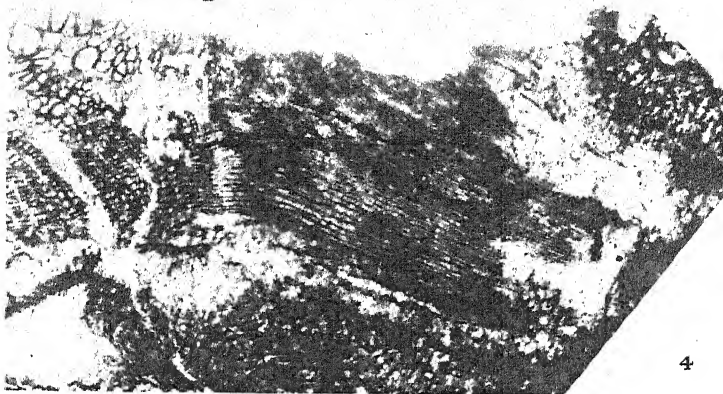


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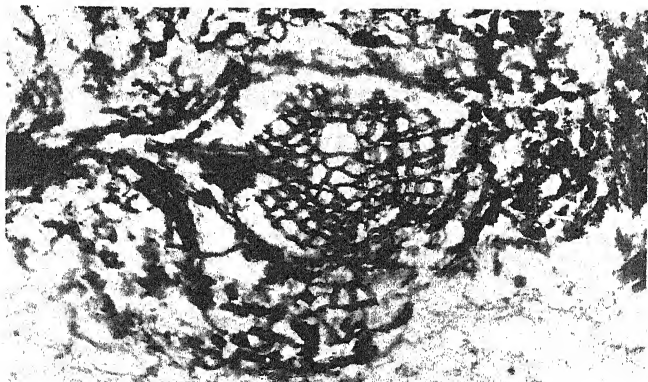


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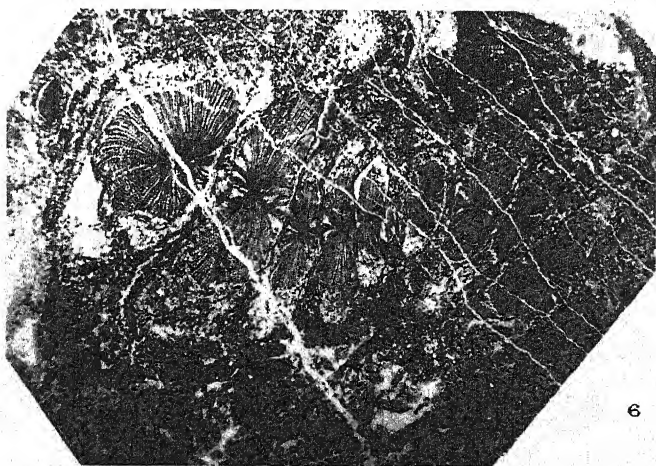
P



4



5



6



# On the Cretaceous Fern *Paradoxopteris* and its Connexion with *Weichselia*.

BY

W. N. EDWARDS.

(*British Museum, Natural History.*)

With Plate XIV and two Figures in the Text.

## CONTENTS.

	PAGE
I. INTRODUCTION . . . . .	318
II. MATERIAL . . . . .	319
III. GENERAL ANATOMY . . . . .	320
IV. HISTOLOGICAL DETAILS . . . . .	321
(i) Xylem . . . . .	321
(ii) Phloem . . . . .	322
(iii) Pericycle . . . . .	323
(iv) Endodermis . . . . .	325
(v) Sclerenchyma . . . . .	325
(vi) Cortex . . . . .	325
(vii) Secretory Canals . . . . .	325
(viii) Crystals . . . . .	326
V. THE FROND GENUS <i>Weichselia</i> . . . . .	327
(i) Constitution of Frond . . . . .	327
(ii) Rachis . . . . .	327
(iii) Arrangement of Pinnules . . . . .	329
(iv) Size and Shape of Pinnules . . . . .	330
(v) Venation . . . . .	331
(vi) Fertile Fronds . . . . .	331
(vii) Cuticle . . . . .	334
(viii) Anatomy . . . . .	335
(ix) Age and Distribution . . . . .	335
VI. SYSTEMATIC POSITION . . . . .	336
VII. SUMMARY . . . . .	337
LITERATURE CITED . . . . .	339
EXPLANATION OF PLATE . . . . .	341

## I. INTRODUCTION.

IN 1911 Professor E. Stromer von Reichenbach discovered, in Cretaceous strata of the Baharia oasis (Libyan desert), some petrified fern stems which were provisionally identified by Schuster as *Osmundites* ((50), pp. 28-31, 34, p. 536, Text-fig. 4). The specimens were described in detail by Hirmer (20) and named *Osmundites* (?) *stromeri*, but later, on account of their aberrant structure, Hirmer (21) instituted the new genus *Paradoxopteris*, which he still included in the family Osmundaceae.

At first glance the transverse section is not unlike that of a mass of leaf-traces of a fossil *Osmunda*, though the bundles are more regularly arranged in rings. Hirmer suggested that these rings of bundles were the leaf-traces, and that the actual stem stele was unknown; he examined about twenty specimens, one of them as much as 20 cm. long, without finding any sign of a central stele. On this view every specimen must have been a mass of leaf-traces, or concrescent leaf-bases which had grown beyond the tip of the stem stele, or else the stele must have dropped out of the stem before petrification.

At the British Association meeting at Cape Town in 1929 I maintained that the curved bundles arranged in rings were not osmundaceous leaf-traces, and suggested that they were meristemes in the highly dissected cylinders of a complex polycyclic stem ((10), p. 384). Further consideration seems to indicate, however, that the structure of *Paradoxopteris* was that of a rachis rather than a main stem.

The late Dr. R. Kidston evidently held the view that the Baharia stems did not belong to the Osmundaceae, as is clear from the following passage in a paper by Seward ((41), p. 137): 'Dr. Kidston recently showed me some petrified stems from Egypt, incorrectly referred by Schuster to the Osmundaceae, which agree very closely with the Belgian *Weichselia* stems described by Bommer. The publication of the results of Dr. Kidston's detailed investigation of the Egyptian *Weichselia* will no doubt furnish more decisive evidence as to the systematic position of the plant.' Kidston never published any account of these stems, and Bommer's work on the structure of *Weichselia* from Belgium, which is further discussed below, did not get beyond the stage of a preliminary note.

In 1930 Schuster, in describing the structure of a *Weichselia* rachis from near Berlin, stated that it was comparable with that of *Paradoxopteris* from Baharia, and renamed Hirmer's species *Weichselia stromeri*.

The present paper deals with certain points in the anatomy of *Paradoxopteris* which have not previously been recorded, and discusses its affinities and probable petiolar nature in relation to the characteristics of the impression-material known as *Weichselia*.

## II. MATERIAL.

The material examined includes :

(i) A specimen in the Geological Department of the British Museum (V. 6124), of unknown provenance, which was transferred from the Botany Department many years ago without any information as to its origin. This specimen is now in three pieces; it is sharply curved almost at right angles, and may have been a fragment from near the base of a rachis. Two old sections cut from it are too thick to be of much use for anatomical study. Further sections have been prepared recently, and an attempt was also made to obtain collodion pulls by Walton's method, after etching with hydrofluoric acid, but, as so frequently happens with silicified material, the results were very poor.

(ii) The opinion of Kidston quoted above suggested that there might be specimens in his collection, and through the kindness of Professor J. M. F. Drummond (formerly of Glasgow) I was able to borrow several slides which were in Kidston's cabinet but had not been fully incorporated in his collection. These slides are numbered 1-8, and the first of them is labelled '*Osmundites* sp. Schuster. Nordwesten der Oase Baharia, Aegypten.' Slide 8 is a longitudinal section, and the remainder transverse, evidently cut from four or five different blocks.

(iii) Professor M. Hirmer very kindly lent me two of his original preparations from Munich, and also a third fine section which he had had specially cut from one of the largest of the Baharia stems (Pl. XIV, Fig. 1). He further gave me a piece of petrified stem which has now been cut and polished and is number V. 21368 in the Geological Department of the British Museum.

The preservation of all this material is very unequal. Thus the specimen figured on Pl. XIV, Fig. 1, shows the general arrangement of the vascular bundles admirably, but the cellular structure is not very well preserved. In one of the Munich sections (Hirmer's Pl. II, Fig. 4) one bundle near the centre is much better preserved than any of the others. All the longitudinal sections leave much to be desired, and it has not been found possible for this reason to give a really complete account of the tissues of the stem.

(iv) Specimens collected by Mr. G. V. Colchester in Darfur (11) include, besides impression material of *Weichselia* which has yielded some useful data, a few fragments with very imperfectly preserved structure which nevertheless suggest comparison with *Paradoxopteris* and with the presumed rachis-fragments of *Weichselia* from other regions.

(v) In addition to *Weichselia* fronds in the British Museum from various localities, I have examined material in the Geological Survey

Museum, London; the Egyptian Survey Museum, Cairo; and the École des Mines, Paris.

For the loan or gift of material, for facilities in examining specimens in other collections, and for assistance in various ways I have to express my thanks to Professor J. M. F. Drummond, Messrs. G. W. Grabham and G. V. Colchester (Khartoum), Professors M. Hirmer and E. Stromer von Reichenbach (Munich), Mr. O. H. Little (Cairo), Dr. R. Crookall (Geol. Survey, London), Professor B. Sahni, and Mr. W. Campbell Smith.

### III. GENERAL ANATOMY.

The polycyclic stelar structure of *Paradoxopteris* has been described by Hirmer (20), (21) as fully as the incomplete material will permit. Essentially the stelar system consists of a number (as many as eleven in one specimen) of more or less regularly concentric circles of U-shaped vertically-running meristeles, alternating with circles of accessory strands which run more or less obliquely through the ground tissue. A transverse section of one of the largest and most regular specimens is shown on Pl. XIV, Fig. 1; in this the division of the meristeles in the outer rings is well shown (Pl. XIV, Fig. 2). In the centre the bundles suggest a spiral arrangement. The accessory strands are typically circular in transverse section; near the point of junction with the meristeles they may be curved or U-shaped, with the concavity facing outwards, i.e. towards the concavity of the meristeles. The outer tissues of the axis have not been preserved.

Although this complicated structure is without exact analogy, as Hirmer has pointed out, among living or fossil ferns, it would seem best to regard these axes as fragments of rachis rather than of a main stem or of a mass of leaf-bases or leaf-traces. The regular arrangement, as well as the structure of the meristeles, suggests a petiole rather than a stem; there is an entire absence of roots; the specimens are round or oval in section, and may be 20 cm. long with no sign of a 'central stele' (which Hirmer presumed was accidentally missing). The nearest approach to the condition of *Paradoxopteris* may perhaps be found in *Angiopteris*, in the petiole of which the bundles are fairly regularly arranged in circles. West ((57), p. 392) states that in the transverse section of the base of the petiole of an old plant there were five separate concentric rings of bundles. There does not, however, appear to be anything corresponding to the accessory strands of *Paradoxopteris*. In the stem of *Angiopteris evecta* there may be as many as nine concentric circles of strands, but they are not quite so regularly arranged as in the petiole, and the meristeles, seen in transverse section, are more irregularly shaped.

Bommer's preliminary account (4) of the structure of *Weichselia* from



the Wealden of Belgium contained more of hypothesis than of detailed description, and the few figures given of what appears to be very indifferently preserved material are insufficient for an exact comparison with *Paradoxopteris*. Schuster (35) has also described the structure of an imperfectly preserved *Weichselia* rachis from Germany, stating that the general structure is completely identical with Bommer's specimens and also with *Paradoxopteris*. It is difficult to appreciate this complete identity from the descriptions and figures of these two authors. Bommer does not mention the rings of accessory strands at all; he states that the inner zones of bundles in both petiole and stem tend to fuse laterally, and that towards the centre of the petiole the form and disposition of the bundles is very irregular. He does not give any essential distinction between the vascular system of the stem and of the petiole. A full description of the Belgian material is much to be desired, together with the evidence for connecting the various organs described by Bommer with *Weichselia*.

Schuster's description includes mention of the accessory strands (Nebenbündel) and agrees more closely with *Paradoxopteris*. He states that an appearance of fusion of the bundles is due to compression. As the rachis he describes actually bore *Weichselia* pinnules, his contribution is particularly valuable. Further details of his specimen are discussed below.

#### IV. HISTOLOGICAL DETAILS.

The examination of additional material has brought to light several points in the anatomy of *Paradoxopteris* which were not recorded by Hirmer, and has also suggested that some modification is necessary in his interpretation of some of the tissues, notably the phloem and pericycle.

##### (i) *Xylem*.

There is little to add to Hirmer's account of the xylem. I have been unable to obtain any satisfactory longitudinal sections through the protoxylem, which appears to be confined to a single endarch group in the concavity of the xylem arc of the horse-shoe-shaped meristeles; in the circular accessory strands it seems to be centrally placed. In bundles which are about to divide there are two protoxylem groups. There is sometimes a certain amount of small-celled parenchyma between the metaxylem elements, especially in the arms of the arc, but apparently no protoxylem. In *Angiopteris* the stem meristeles contain several protoxylem groups which may be mesarch or endarch, but in the petiolar bundles there is a tendency to form a single endarch group ((42), p. 520).

The finely scalariform thickenings of the tracheides have already been figured by Hirmer ((20), Pl. V, Fig. 4 H, 4 I). Tyloses occur in some of the metaxylem elements (Text-fig. 1 A).

(ii) *Phloem.*

The phloem was described by Hirmer ((20), p. 14, Pl. IV, Fig. 4 E, 4 F) as a layer of large rectangular cells, the radial walls of which were about three times as long as the tangential. This layer is a characteristic feature

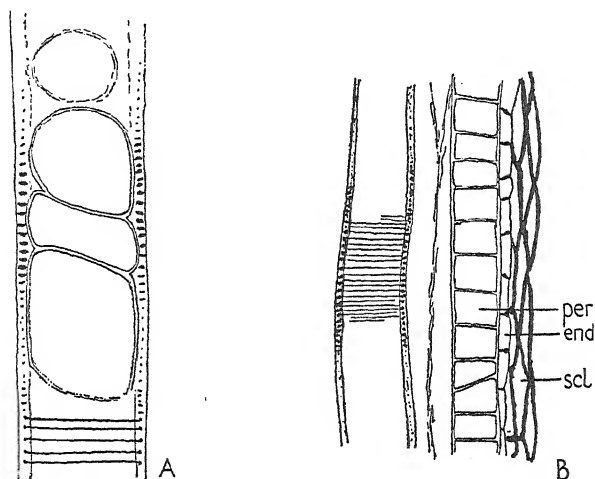


FIG. 1. *Paradoxopteris stromeri* Hirmer. Longitudinal sections. A. Tracheide with tyloses.  $\times$  c. 100. (V. 6124d.) B. Rectangular pericycle cells (*per.*); endodermis (*end.*); sclerenchyma sheath (*scl.*).  $\times$  c. 66. (V. 6124c.)

of the vascular bundles of *Paradoxopteris*, but it is very different from any known phloem, and is described below as a pericycle. The tissue marked in Hirmer's figures 4 E and 4 F as xylem parenchyma may be partly phloem parenchyma, but the sieve-tubes are usually crushed or not preserved. In one fairly well-preserved bundle of one of the Munich specimens (kindly lent me by Professor Hirmer) the sieve-tubes are, however, very clearly seen in transverse section. This bundle is the one nearest the top of the figure on the right-hand side of Hirmer's Pl. IV, Fig. 4 D, and is here shown enlarged on Pl. XIV, Fig. 3. The belt of oval sieve-tubes can be seen separated from the metaxylem only by a few parenchymatous cells; the tissue which I consider to be the pericycle is here torn and almost completely destroyed, but a few fragments of cell-wall remain attached to what appears to be the endodermis, and show that there was formerly a layer of cells in this position. In one of Kidston's slides, in which the phloem is well preserved, the relics of the pericycle are more clearly seen (Text-fig. 2). Whenever the sieve-tubes are preserved they are of a large size, and round or oval in transverse section; similar tubes occur in various ferns, but it may be noted that in *Angiopteris* the petiolar bundles are characterized by the large size of the sieve-tubes ((40), p. 319).

(iii) *Pericycle*.

As stated above, the pericycle in *Paradoxopteris* is an extremely well-developed layer of large rectangular cells, which are sometimes almost square in section, and sometimes much elongated radially, forming a regular

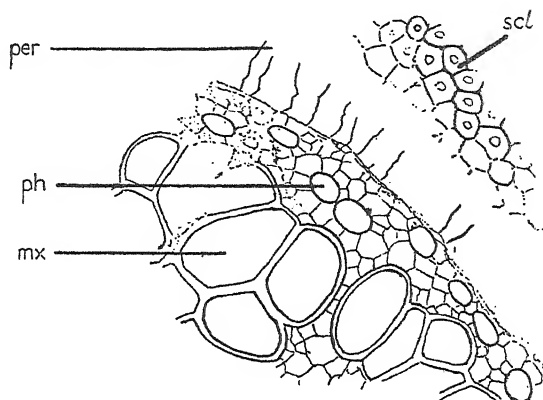


FIG. 2. *Paradoxopteris stromeri* Hirmer. Transverse section of part of a bundle; *mx*. metaxylem; *ph*. phloem; *per*. torn pericycle cells; *scl*. sclerenchyma.  $\times$  c. 75. (Kidston unnumbered coll., slide 3.)

palisade tissue (Pl. XIV, Figs. 4-6). They are apparently never longitudinally elongated (cf. Text-fig. 1 B). The large cells, which sometimes extend almost completely round the bundle, become smaller towards the centre of the concavity on the inner side, and in the neighbourhood of the protoxylem cannot definitely be distinguished. In the circular accessory strands the pericycle is usually very well marked (Pl. XIV, Figs. 5, 6). There is considerable variation in the size of the cells, and they are sometimes little or no longer than the adjoining sieve-tubes. Though usually only one cell in depth, the palisade pericycle may in places be divided by tangential walls to form a layer two or three cells deep.

A somewhat large-celled pericycle is not uncommon among recent ferns, though I know of no case in which it forms such a very definite layer of usually rectangular cells as in *Paradoxopteris*. Thus in the petiole of *Loxsonia*, according to Gwynne-Vaughan ((18), p. 83, Pl. III, Figs. 5, 6), 'the pericycle consists of two or three layers, except in the median region, where only one is present; on the outside of the flanks of the horse-shoe its cells are considerably larger than elsewhere'. A somewhat similar pericycle occurs in *Davallia speluncae* (Gwynne-Vaughan (19), Pl. XXXV, Fig. 26). The stem of *Schizaea malaccana* has rather large pericycle cells, which occasionally divide by additional walls, either radial or tangential ((51), Pl. XXVI, Fig. 13). According to Stopes and Fujii ((49), p. 12), in some specimens of *Aneimia* and of *Dicksonia* the pericycle cells are markedly large. Poirault ((81), p. 135) describes a large-celled pericycle in

the root of *Balantium antarcticum*. Thomaë ((52), Pl. VIII, Fig. 7) figures a large-celled pericycle, in which some of the cells are almost rectangular, and some are radially elongated, in *Hemitelia spectabilis*, and a drawing by Schütze ((36), p. 355, Fig. 10) of part of the leaf-bundle of *Cyathea usambarensis* suggests a similar development. I have observed large pericycle cells in the petioles of *Gleichenia flabellata* and *Trichomanes radicans*, and in the latter there is sometimes a tendency to radial elongation.

Among fossil ferns, however, there are several instances of a well-developed palisade pericycle, much more closely resembling that of *Paradoxopteris*. (a) In *Fasciostelopteris tansleii* Stopes and Fujii ((49), p. 10, Pl. I, Fig. 7), from the Upper Cretaceous of Hokkaido, Japan, a large-celled palisade pericycle surrounds each meristeles, the elements being particularly large at the curves of the stele. (b) There is a suggestion of a large-celled pericycle in some of the meristeles of *Yezopteris polycycloides* Ogura ((29), p. 381) from Hokkaido, as seen in sections in the Geological Department of the British Museum, but the preservation is too poor for one to feel certain. The opinion may here be hazarded, however, that *Yezopteris* and the very fragmentary *Fasciostelopteris* just mentioned may be either related or 'perhaps even identical. (c) In *Cyathorachis fujiana* Ogura, also from the Cretaceous of Japan, 'pericyclic cells are large and thin-walled, but sometimes are extremely long in the radial direction and thick-walled; this is the most conspicuous feature of the present fossil' ((28), p. 371). As this feature was not figured by Professor Ogura, I give here a photograph from a slide presented by him to the British Museum (Pl. XIV, Fig 9). (d) Stenzel ((44), p. 12, Pl. II, Figs. 7, 8) figured a palisade pericycle of very elongated cells, usually one cell deep, but sometimes of two or three cells diminishing in size, in *Caulopteris* [= *Protopteris*] *arborescens*<sup>1</sup> from Kamenz in Saxony (geological formation unknown, perhaps Cretaceous or Tertiary). (e) On the inner side of the stem stele of *Protopteris cottaeana* Presl (in Sternberg (45), p. 170) there is a palisade layer marked 'c' in Corda's figures ((45), Pl. LXVII, Figs. 2, 3; (7), Pl. XLIX, Figs. 6, 7), which Corda interpreted as a phloem band, but which seems to me to be more probably a pericycle. There is a fragment of Corda's original specimen, with slides cut from it, in the Geological Department of the British Museum and, though the preservation is imperfect, it seems possible that the phloem is represented by the layer of rather crushed cells between the xylem and the palisade tissue. Further, although this palisade tissue is developed mainly on the inner side, it also occurs in

<sup>1</sup> This species has recently been placed together with *Rhizodendron oppoliense* Goeppert in a new genus, *Stenzeliella*, by Zalesky ((59), p. 16), but the new name is superfluous, and if the two species are congeneric and distinguishable from *Protopteris*, Goeppert's name *Rhizodendron* must be used.

places on the outer side of the xylem band, as in *Fasciosteleopteris* and *Protopteris arborescens*, and a longitudinal section suggests that the cells are only elongated in a radial direction.

The above fossils are all considered to belong to, or to be related to, the Cyatheaceae, and I am not aware of the presence of a palisade pericycle in any other fossil ferns. In view of the occurrence of a large-celled pericycle in recent ferns belonging to various families, the character may not be of any systematic importance; it may be a xerophytic adaptation connected with water-storage.

(iv) *Endodermis*.

Immediately outside the pericycle it is frequently possible, whenever the preservation is good enough, to detect a layer of cells which must represent the endodermis. This layer is particularly clearly seen in the meristele figured on Pl. XIV, Fig. 3, in which, however, both the pericycle and the sclerenchymatous sheath have been torn (see also Text-fig. 1 B). In the case of a fossil it is of course impossible to study the endodermis as in a living plant, and one can only point out the apparent presence of an endodermis in *Paradoxopteris*. The petiolar bundles of living Marattiaceae are characterized by the absence of an endodermis, but this may be of functional rather than systematic importance.

(v) *Sclerenchyma*.

All the bundles are surrounded by a sclerenchymatous sheath of fibres, usually from two or three to five or six cells in width, but sometimes as much as ten or more. Living members of the Marattiaceae are on the whole characterized by an absence of sclerenchyma, though it occurs in the petiole of *Angiopteris*, where it does not surround each separate bundle, but forms a band just below the epidermis. Sclerenchyma occurs in the fossil Psaroniae, which are considered to have marattiaceous affinities.

(vi) *Cortex*.

The ground tissue consists of rounded and usually isodiametrical parenchymatous cells, which sometimes contain a light or dark brown substance. Light brown globules are particularly well shown in certain cells of Kidston's slide no. 3; they are suggestive of tannin cells, such as occur commonly in the Marattiaceae. The outer cortex and epidermis are not preserved in any specimen I have seen.

(vii) *Secretory canals*.

In addition to the presumed tannin-cells, the cortex contains secretory canals (Pl. XIV, Fig. 7) which alternate regularly with the curved meristemes, and are usually slightly external to them. They may be compared

with the well-known mucilage canals of the Marattiaceae, and, though the point is not easy to determine in petrified material which is imperfectly preserved, epithelial cells appear to be absent, as in the adult canals of the Marattiaceae. According to West (56), who has given the most recent review of secretory tissues in this family, the canals are of lysigenous origin, but in the petiole of *A. evecta* they may be lysigenous or schizo-lysigenous. He states (p. 415) that 'the adult mucilage-ducts, which frequently branch and anastomose, pursue a very irregular course through the ground tissue'. Thomae ((52), p. 133, Pl. VI, Fig. 17), however, states that in *A. evecta* the canals are arranged fairly regularly in circles, and his diagrammatic figure of a transverse section of a rachis shows them more or less alternating with the vascular bundles. This is obviously comparable to the arrangement in *Paradoxopteris*.

Schuster ((35), p. 65) describes in a petiole of *Weichselia* 'zahlreichen schizolysigenen Sekretgängen in parallelen Reihen, die mit den Blattspurbündeln alternieren und von einem Kranz von 12–14 kleinen isodiametrischen Epithelzellen umgeben sind'. This is an important piece of evidence in connecting *Paradoxopteris* with *Weichselia*. Schuster accepts the connexion between the two genera, but states that *P. stromeri* differs from *W. reticulata* in the absence of secretory canals and crystal cells. Neither of these distinctions holds (see also below). The canals are not mentioned in Hirmer's account (20), but can be clearly seen in several places in his Figure 4 D, Pl. IV, and they are present in all specimens which I have examined. The cortical cells surrounding the canals are often arranged concentrically, diminishing in size towards the centre, and Schuster's epithelial layer may be only a ring of cortical cells; his material may of course be better preserved than the Egyptian specimens, but his Fig. 5, Pl. IX, is by no means clear. Secretory canals also occur in Bommer's Belgian material.

#### (viii) *Crystals*.

Schuster described and figured ((35), p. 66, Pl. IX, Fig. 7) crystals of quartz, usually quadratic in section, in some of the parenchyma cells of a partly-silicified rachis of *Weichselia* from Germany. These crystals were stated to be embedded in quartz and to show the same interference tints and extinction as the surrounding mass. Schuster therefore considered that they represented original crystals in the plant cells which had been replaced by quartz. Several of the slides of *Paradoxopteris* in the Kidston collection also show numerous quartz crystals (Pl. XIV, Fig. 8), but they are nearly always hexagonal in section, and they occur not only in parenchyma cells, but also in the xylem and even in intercellular spaces. Sometimes there is a single crystal in each cell, in optical continuity with the surrounding chalcedonic infilling, as in Schuster's *Weichselia*, but some-

times the whole of a large tracheide is filled with crystals. Usually each crystal has a dark central nucleus, and sometimes the cell-cavities are lined with small dark bodies round which crystals have grown out into the cell. It seems clear that there is no question here of replacement by the quartz, but that the crystals must have formed round the nuclei (the nature of which has not been determined) at an early stage in the silicification of the plant. The crystals in Schuster's specimen may also have been similarly formed in the course of petrification; it certainly seems most unlikely that they are replacements of, for example, oxalates of calcium and magnesium such as occur commonly in the Marattiaceae. Whatever their origin, their presence cannot be used, as Schuster suggests ((35), p. 75), as a point of distinction between *Weichselia* and *Paradoxopteris stromeri*. Poirault (31, p. 240) describes the occurrence of 'nodules siliceux' in the Marattiaceae, but they seem to be confined to the epidermal cells.

#### V. THE FROND GENUS *WEICHSELIA*.

One of the most widely spread genera of Lower Cretaceous ferns is *Weichselia* Stiehler (46), the type (and perhaps the only) species, *W. reticulata*, having been originally described by Stokes and Webb in 1824 as *Pecopteris reticulata*. The early records and synonymy of this species have been fully dealt with by Seward (37), Gothan (16), and Stopes (48). Numerous papers dealing with *Weichselia* have appeared since, though its affinities still remain uncertain. In the following critical review of the genus it will be convenient to consider the various characters separately.

##### (i) *Constitution of frond.*

Most authors are agreed that the fronds of *Weichselia* were bipinnate, though Zeiller (61) thought that they might be tripinnate in the Peruvian material, owing to the large size of some of the rachis fragments. However, no tripinnate fronds have so far actually been observed. Bommer's hypothetical reconstruction (4) of the frond as pedate has never been confirmed, and is rendered even less likely by Gothan's discovery of the impression of a *Weichselia* plant showing a ring of bipinnate fronds attached to a short stout stem ((17), p. 772). Lipps ((24), p. 342) describes but does not illustrate a frond with a bifurcating rachis; his description is not very clear, and the specimen may have been an abnormality, if indeed it was a *Weichselia* at all. It seems that at present no subdivision of the genus can be made on the basis of frond form.

##### (ii) *Rachis.*

The most detailed description of the rachis is that given by Zeiller ((60, 61), p. 657), founded on the Peruvian material named by him *Weichselia*

*peruviana* (Neumann). Zeiller suggested that the ridges were probably due to sub-epidermal sclerenchymatous strands, which only appeared as a result of compression or desiccation, the surface otherwise remaining smooth. Rachis fragments from Darfur collected by Mr. G. V. Colchester are mostly uncompressed casts or moulds, and hence are not noticeably ribbed. The fine striation, possibly due to longitudinally arranged epidermal cells, is not likely to be seen in specimens preserved in a coarse matrix. One of the casts from Darfur (11), probably of a *Weichselia* rachis, resembles Zeiller's Pl. XXI, Fig. 11. A recently figured specimen from Syria ((9), Pl. VI, Fig. 3) agrees exactly with Zeiller's description and figures (especially his Pl. XXI, Fig. 6). A similar specimen, though not so well preserved, was recorded in the same paper from Transjordan. Excellent illustrations of the rachis from the Wealden of Féron and Montfaux, France, have been given by Carpentier ((5), Pl. XII, Figs. 11, 12). There is no reason to doubt that these belong to *W. reticulata*.

Fritel has recorded ((14), p. 74; (15), p. 315) fragments of the rachis of *Weichselia* from the Nubian Sandstone of Assouan, but his figures do not show clearly the characteristic ribbing and striation first described by Zeiller. In view of the complete absence of pinnules, and in spite of the apparent occurrence of rachis fragments with two rows of pinna-scars (which are not figured), this identification must remain somewhat uncertain, as Fritel himself admitted. The flora is considered to be middle Cretaceous, and the association of *Weichselia* with numerous dicotyledons would, if confirmed, be of considerable interest.<sup>1</sup>

The pinna-bearing rachis of specimens of *W. reticulata* from English Wealden beds is sometimes obscurely ridged, but in Europe the broader specimens, which probably come from the base of the rachis where no pinnæ were borne, may often have been overlooked or recorded under other names. The Peruvian specimens were first described as *Equisetites*, and I believe that a specimen from the Klin sandstone figured by Trautschold ((53), Pl. XVIII, Fig. 1) as *Equisetites* sp. is a piece of *Weichselia* rachis. Figure 3 ('*Equisetites* sp.') and Fig. 8 ('*Calamites* sp.') on the same plate may also be *Weichselia*, although the former, if natural size, is exceptionally wide.

One of the specimens figured by Stiehler ((46), Pl. XIV, Fig. D) as *Pandanus similidae* might possibly belong to *Weichselia*, and it is noteworthy that in recording *W. reticulata* from Venezuela, Schlagintweit (33) speaks of its association with '*Equisetites*? . . . wie mir solches aus Peru ebenfalls bekannt ist'.

<sup>1</sup> In his second paper (15) Fritel lays stress on the relative importance of the cycads in this flora, mainly on the grounds of supposed pith-casts which would be best regarded as unidentifiable. He compares one of them to the Lower Greensand *Benstedtia*, which he calls a cycad, but which was long since shown to be a conifer.



It is unfortunate that the material examined by Lipps (24) is inadequately illustrated. He states that the main rachis of one of his specimens had 'eine hohle Rinne' on the upper side (p. 340), and his only other reference to the surface of the rachis is in the description of a specimen which he calls Type c (p. 344): 'Die Sekundärspindeln tragen auf ihrer Rückseite einen feinen, erhabenen Längsstreifen, der übrigens auch bei andern *Weichselia*-Typen vorkommt'.

Bommer (4) describes the surface of the rachis in his Belgian material as very rugose, owing to the presence of numerous small protuberances. This condition does not seem to have been observed at all in any specimens of *Weichselia* from other parts of the world, and the matter evidently requires further investigation.

(iii) *Arrangement of pinnules.*

The pinnules are usually at right angles to the rachis, or nearly so, but may make an angle of about  $45^{\circ}$ . The angle is sometimes more acute towards the distal end of the pinna. Though usually closely set, the pinnules may be separated. In these, as in some other characters (e.g. size and shape), one finds the same range of variation in material from a number of different localities, and there does not seem to be any constant specific difference.

The two rows of pinnules, one on each side of the rachis, are very often inclined towards each other, and this, the well-known 'butterfly position', is probably a natural feature. Gothan ((16), p. 6) suggested that it was only found in specimens preserved in sandstone, and hence was perhaps due to rapid drying, but Lipps ((24), p. 341) and Hirmer ((20), p. 4) have pointed out that it occurs also in fine-grained clays and shales. The apparently unilateral arrangement (cf. one of the Syrian specimens, (9), Pl. VI, Fig. 2) is probably due, as Hirmer points out, to an original 'butterfly position', and the same arrangement is sometimes found in English Wealden and in Transjordanian material. Obviously the two rows of pinnules might often become flattened together in fossilization, and the inclination of the pinnule-rows was probably a universal character.

The reflexed basal pinnules serve, in Berry's opinion, to distinguish the Peruvian material from *W. reticulata*. *W. peruviana* was founded on the nature of the supposed fertile fronds, although Zeiller added ((61), p. 663) that perhaps it also differed in the arrangement of the basal pinnules. However, Lipps ((24), pp. 342-4) describes reflexed basal pinnules in more than one of the 'types' which he endeavours to distinguish in the Barremian *Weichselia* fronds of Hildesheim. Further, a specimen from Darfur (Brit. Mus. Nat. Hist. V. 21686) shows basal pinnules which are strongly reflexed, and a specimen from Transjordan (B.M.N.H. V. 20503) shows them slightly reflexed. Some of the drawings of

Belgian specimens given by Seward ((38), esp. Pl. I, Fig. 3) suggest reflexion, and probably this character has frequently been overlooked, or else has not been preserved. I have examined numerous English specimens, both in the British Museum and the Geological Survey Museum, without finding one in which the basal pinnules were clearly preserved. Prof. Berry himself has suggested ((1), p. 54) that *Gleichenia* (?) *gilbert-thomsoni* Font. is possibly a *Weichselia*, and it is noteworthy that the figures of this frond from California ((55), Pl. LXVI, Fig. 11) and from British Columbia ((30), Pl. IX) both show reflexed basal pinnules. Some at least of Traut-schold's *Asplenites klinensis* are *Weichselia reticulata*, but whether this name should be applied to his Pl. XX, Figs. 2, 3 (53), which show slightly reflexed pinnules, is uncertain, though quite possible. A specimen of *W. reticulata* from Siberia figured by Kryshstofovich ((23), Pl. LX) appears to have reflexed basal pinnules.

The occurrence of pinnules on the main rachis, at the distal end, has been recorded by Berry (2).

(iv) *Size and shape of pinnules.*

Much of the variation in the pinnules is due to difference in position on the pinna, and it is recognized that the distal pinnules are more pointed and more deltoid than the others. The variation in the fragmentary Belgian material has been described by Seward (38), who has also shown that the pinnae may terminate in long, sometimes lobed, segments. In a series of specimens from the Wealden of Shepherd's Chine, Isle of Wight, in the Geological Survey Museum, there is the same variation. Specimen no J.R. 3815 shows a lobed terminal segment, about 9 mm. long, and another fragment on the same block shows a slight contraction at the base of the pinnules similar to that of a specimen from Syria ((9), p. 397). A specimen from the Wealden of Atherfield in the Survey Museum (no. 48481) has pinnules 8 mm. long. In 1926 I recorded specimens from Darfur with pinnules from 6 to 9 mm. long, and Mr. G. V. Colchester has since collected further material from the same locality in which some pinnules are only 3 mm. and others as much as 17 mm. long. Lipps ((24) p. 343) speaks of pinnules 'at least' 9 mm. long from Germany, and one of the Syrian specimens (V. 20486) is 10.5 mm. long. The long form of pinnule is therefore much commoner in localities where short pinnules also occur, or even on the same frond ((11), p. 409), than Nathorst realized when he provisionally founded a species (*W. erratica*) on a few pinnules 9 mm. long from the Swedish Ryedal Sandstone ((26), p. 24), and this name obviously cannot stand.

Schuster (35), in describing a Holma-sandstone erratic containing *Weichselia* with pinnules 10–11 mm. long (though his Pl. VIII, Fig. 1, suggests that some of them may have been shorter), reduces *W. erratica* Nath.

to a variety of *W. reticulata*, retaining the former name on the ground of the 'constant' large size of the pinnules. In other localities the size is certainly not constant, and it is difficult to imagine how the constancy of such a character can be deduced from a very few pinnules preserved on a very fragmentary frond.

Lipps speaks of the reflexed basal pinnules as being somewhat spoon-shaped, and contracted at the base. His figures, unfortunately, are mere smudges, and the only analogous case I know is a rather poor figure of *Asplenites klinensis* Trautschold ((53), Pl. XX, Fig. 2), which, as stated above, may be *W. reticulata*. Lipps, who believes that *Weichselia* is related to *Gleichenia*, states that such basal pinnules also occur in some recent Gleicheniaceae. A modification of the basal pinnule is also to be seen in *Pecopteris geyleyriana* Nathorst (25) from Japan, which is referred by Seward to *Cladophlebis dunkeri*. The specimens referred to *P. geyleyriana* by Ward ((54), Pl. CLX, Fig. 9-13) are probably *W. reticulata*.

(v) *Venation*.

The characteristic reticulate venation is unfortunately not always very well preserved, so that although one may be able to recognize its presence a photograph or an accurate drawing of all the veins is usually difficult to obtain. Many of the published drawings are distinctly diagrammatic, and the best figures are those of Hirmer ((20), Pl. I). There is some variation, obviously unimportant, in the size of the areolae between the midrib and the margin. In Hirmer's photographs the areolae near the margin are about the same size as, or slightly smaller than, the adjoining ones, but in a drawing given by Berry ((1), Pl. II, Fig. 2) the outermost areolae are all elongated. This is a very common appearance, but may not be original, for a pinnule from Transjordan (V. 20499) appears to have elongated outer areolae along one margin only. Differences in preservation account for a good deal of the variety. One of the fragments figured by Steinmann ((43), Fig. 107, p. 102), which shows simple or forking secondary veins, with only rare anastomoses, must be a case merely of poor preservation.

The general direction of the veins may be either at right angles to the midrib or (as in Hirmer's figures) at an acute angle. The direction is often at right angles in the shorter pinnules, and at an acute angle in the longer ones, but I do not think any constant distinction can be made. In some pinnules the direction is at right angles near the base, becoming more inclined towards the apex.

(vi) *Fertile fronds*.

If all the supposed cases of fertile *W. reticulata* could be substantiated, the 'species' would have to be split into half a dozen genera, belonging to

as many different families. In many cases there is, however, to say the least, an element of doubt.

(a) Trautschold ((53), Pl. XX, Fig. 7) figured a supposed fertile fragment of his *A. klinensis* (= *W. reticulata*) with linear sori inclined to the midrib. In poorly preserved impressions, however, especially when a little carbonaceous matter is present, a precisely similar appearance is frequently produced owing to the general direction of the veins, mentioned above. Such an appearance can be seen on some of the long pinnules of the Syrian specimens, while other pinnules on the same pinna are distinctly reticulate. Trautschold's case can therefore be dismissed at once.

(b) Nathorst ((25), Pl. IV, Fig. 3) figured a small fragment of what he thought might be a fertile frond of *Pecopteris geyleriana* from Japan. This specimen was referred to *W. reticulata* by Seward ((37), p. 116), but neither as a fertile frond nor as a *Weichselia* is it very convincing.

(c) Gothan ((16), p. 12, Fig. 5) tentatively suggested that a specimen from Quedlinburg with an apparently thickened margin might be fertile; the evidence is so slender that this specimen need not be considered further.

(d) Neumann in 1907 figured some fragments of fronds, supposedly of *W. reticulata*, showing round 'sori' lying close to the midrib. No structural details were given. The drawings are very poor, and if the venation is correctly drawn, the specimens cannot be *Weichselia*. However, Zeiller, in dealing with the Peruvian material (61), accepted Neumann's conclusions, and on the strength of the difference between these fertile fronds and those described by Bommer from Belgium (see below) instituted a new species, *W. peruviana* (Neumann). The specific name was adopted from Neumann's *Equisetites peruanus*, which was in reality the rachis of *Weichselia*. It is to be noted (1) that Neumann's and Zeiller's figures do not entirely support each other: in the latter's photograph the round impressions are relatively very much larger; (2) that Zeiller does not definitely state that these fertile fronds also showed reticulate venation. The possibility that they may have belonged to some other plant does not seem to be excluded, even if the round markings were actually sori.

I recently had an opportunity of examining Zeiller's Peruvian material in the École des Mines at Paris. The preservation was exceedingly poor, and in some cases it certainly seemed that the supposed sori were merely accidental markings. A few of the specimens were undoubtedly fertile fronds, but I could see no sound reason for referring them to *Weichselia*; they may have been *Matonidium* or *Klukia*, but for the present would best be regarded as unidentifiable.

Berry, who has examined a large amount of material from Peru, has seen no trace of fructifications, and he considers that Neumann's fertile pinnules were imaginary (Berry (2), p. 4). He still retains *W. peruviana*

as a distinct species, however, falling back on Zeiller's second line of defence, the reflexed basal pinnules. Steinmann ((43), p. 101) expressed some uncertainty as to the validity of this distinction, and, as we have seen above, it will not hold. *W. peruviana* must, therefore, be regarded as a synonym of *W. reticulata*.

(e) In 1900 Seward described, under the name of *Conites minuta*, some small spherical objects from the Wealden of Bernissart. They were about 4 mm. in diameter, and apparently attached to an axis, but though their botanical affinities were doubtful, the author thought that there was some resemblance to the female cone of a Conifer. These fossils were described by Bommer (4) as the synangia of *Weichselia*. There may be evidence for this ascription, but it does not appear in Bommer's paper. The specimens were all very fragmentary, and it is not even clear whether Bommer's criterion of the presence of rugosities on the rachis is intended to apply to these fragments. (As stated above, no other worker has described a rugose rachis in *Weichselia*.) The objects which Seward ((38), Pl. IV, Fig. 62) thought might be cone-scales are interpreted by Bommer as sporangia, and he states that they had an incomplete annulus (but does not figure it). One is led to the conclusion that the material must be very unsatisfactory, and that perhaps it would be safer, while awaiting a more complete account of Bommer's specimens, to revert to the non-committal term *Conites minuta*.

A few years ago Fraipont (13) brought forward evidence for regarding *C. minuta* as the male cone of a Conifer; on macerating a specimen from Charleroi he obtained numerous spores, and also, from a fragment of the stalk, tracheides with bordered pits. Fraipont proposed the name *Smeystersia minuta* (Sew.) for his specimen, and concluded that it was specifically identical with the cones from Bernissart. A final decision does not yet seem possible, but it would be rash to assume the identity of the Charleroi and Bernissart specimens.

(f) A specimen which bears a considerable resemblance to *C. minuta* was figured by Nathorst ((26), Pl. I, Figs. 5-7) from a boulder of Ryedal sandstone, which also contained fragments of *Weichselia*. Nathorst did not name the specimen and, though he compares it principally with the fertile frond of *Onoclea*, suggests that it may not even be a fern. Bommer noted the resemblance to the Belgian specimens, and apparently founded his restoration of a fertile *Weichselia* on Nathorst's Fig. 5. Bommer states that Nathorst 'les avait considérées comme pouvant représenter la fructification de cette Fougère', but I cannot find any foundation for this statement in Nathorst's paper. Schuster ((35), p. 72) also considers it highly probable that this specimen was a fertile fragment of *Weichselia*, and states that a similar specimen, from the Holma sandstone of Rixdorf (Lower Cretaceous), is in the Geological-Palaeontological Museum at Berlin.

(g) Another fructification, said to be similar, is from the Barremian of Hildesheim (24). Unfortunately the figure of this specimen (Fig. 10, p. 340) shows nothing whatever, and the description does not help very much. Lipps states (p. 345) that the fructifications resemble Bommer's 'synangia', and that they are flattened globular bodies, 2–3 mm. in diameter, borne in two rows, or paired, on curved axes. It is not certain whether these somewhat obscure fructifications, which occur in Lower Cretaceous beds of Belgium, Sweden, Germany, and perhaps Peru (see below), are of the same type, but they are the only specimens so far recorded which seem at all likely to have been the fructifications of *Weichselia*.<sup>1</sup>

(h) Lipps also describes another supposed occurrence of fructifications ((24), p. 342), stating that from the folds of an aplebia he isolated flattened *sporangia* (not synangia) of *Weichselia*. Although he suggests that the aplebia probably wrapped round an undeveloped fertile frond, this occurrence can scarcely be reconciled with the one just adduced, and obviously requires confirmation and illustration, as also does his statement that *Weichselia* bore aplebiae. Ripe detached fructifications might of course be found lodged in an aplebia or in a rachis-base, but would not afford any conclusive evidence in such a case as that of *Weichselia*, and might even be derived from a different plant.

(i) One may tentatively suggest that a specimen from Peru described by Steinmann as a new generic type (*Peruviophyllum minutifolium* Steinmann ((43), p. 105, Fig. 113) may perhaps be a fertile frond of *Weichselia*, for it seems to resemble the type of fructification first described by Nathorst, and which is associated with *Weichselia* in various parts of Europe.

Steinmann described his specimens as bearing diminutive egg-shaped pinnules which did not show the nervation; he evidently did not suspect that the specimen might have been a fertile frond. The rachis of *Peruviophyllum* in Steinmann's figure resembles that of *Weichselia*.

(vii) *Cuticle*.

The epidermal structure of some Belgian fronds has been investigated by Florin (12), but as the cuticle is very rarely preserved there is no evidence as to the possible variation in fronds from other regions. I have obtained a few cuticular fragments by macerating some English Wealden specimens, and the stomata appear to agree with Florin's figures, but the preservation is not so good. Florin concluded, apropos of the suggestion made in various quarters that *Weichselia* might not be a fern at all, that there was nothing in the epidermal structure which was inconsistent with a reference to the ferns, and that the stomata showed a marked xerophilous character. He found no trace of hairs on the epidermis.

<sup>1</sup> According to Schuster (35), Lipps has in hand a complete account of his fertile frond of *Weichselia*, including a recognition of the spores.

(viii) *Anatomy.*

The internal structure of the rachis has already been discussed above in connexion with *Paradoxopteris*, and Zeiller's inferences from rachis impressions have also been mentioned. The ribbing in these impressions might be due either to sub-epidermal sclerenchymatous strands as suggested by Zeiller, or to the sclerenchyma of the outermost ring of petiolar bundles.

(ix) *Age and distribution.*

*Weichselia* has been recorded from Lower Cretaceous beds (principally Neocomian and Barremian) of central and northern Europe, Russia, Siberia, Japan, Syria, Transjordan, Egypt, Darfur, Venezuela, Peru, the United States, and Canada.

In Germany it occurs, though rarely, as high as the Gault (16); in England it has already been found in the Aptian (Lower Greensand (48)), and I am now able to record it also from the Albion (Upper Greensand). A block of 'cowstone' with *Pityostrobus*, collected by Mr. H. C. W. Davis at Humble Point, Seaton, Devon (B.M.N.H. V. 20181), contained typical fragments of *W. reticulata*, with some of the fronds in the inclined ('butterfly') position.

Stromer's material from Egypt (Baharia) is regarded as of lower Cenomanian age; in some other cases (e.g. occurrences in the Nubian Sandstone) the exact age is uncertain. When in Cairo in 1929 I had an opportunity of examining the specimen from the desert east of the Nile, between Assuan and Wadi Halfa, described as ?*Weichselia* sp. by Seward (39). This poorly preserved fern frond has some resemblance in habit to a very small-pinnuled *Weichselia*, but apart from an occasional indistinct midrib the venation was not preserved at all, and the specimen seems to be indeterminable.

Kryshtofovich (23) has figured *W. reticulata* from Siberia (Ussuriland), in beds for which an Aptian age is suggested, associated with a presumed monocotyledon (*Pandanophyllum ahnertii*). One of the records from Japan (see above, p. 332) is not absolutely certain, but Yabe ((58), p. 89) records *W. reticulata* from the lower Cretaceous (Inkstone series) of Nagato.

Gothan's suggestion that *Weichselia* was probably a strand plant is supported by Carpentier (6), who remarks on the frequent occurrence of fragments of the frond in marine deposits where other plants are absent or rare, and Schuster (35) describes the flora associated with *Weichselia* in the Holm Sandstone as a xerophytic strand-flora.

## VI. SYSTEMATIC POSITION.

The affinities of *Weichselia* have for many years been subject to discussion. Nathorst and others suggested that it might not even be a fern at all, but the anatomical details which have since come to light dispose of any doubt on this point. The frond characters alone offer insufficient guidance: Lipps, for example, suggested an affinity with Gleicheniaceae, apparently on grounds of the general appearance of the frond, and the presence of reflexed basal pinnules; the vascular structure, however, proves to be totally unlike that of the Gleicheniaceae. The fertile fronds are still uncertain, and even if the '*Conites minuta*' type of fructification belongs to *Weichselia*, details of synangial or sporangial structure are still lacking. Bommer considered that the sum of characters recalled the organization of the Marattiaceae, but that there was an even closer resemblance to the Matoniaceae in the structure of stem and petioles, the mode of division of the fronds, and up to a point in the organization of the sori. Bommer's reconstruction of frond habit is, however, probably incorrect, and the structure of the presumed sori is insufficiently known. Zeiller (60, 61) favoured an affinity with the Marattiaceae rather than the Matoniaceae.

The internal anatomy does not at present offer any conclusive indication of family affinity. The original reference of *Paradoxopteris* to the Osmundaceae cannot be maintained, and Hirmer himself admitted that the stelar structure was quite anomalous. Polycyclus is a phenomenon which may arise in different groups of ferns, as a response to the need for a more highly developed conducting and water-storing tissue, in connexion with increase in size and the crowding of fronds on the stem. Members of the Polypodiaceae (such as *Platyserium*), Cyatheaceae, Marattiaceae, and Matoniaceae, as well as the fossil Psaroniae, exhibit polycyclic stem steles, and some members of the first three of these families have polycyclic petioles. In stelar structure *Angiopteris* seems to afford the nearest approach to *Paradoxopteris*, and some anatomical details also support the idea of an affinity with the Marattiaceae, particularly the presence and arrangement of the mucilage canals. The presence of tannin cells and the large sieve-tubes may also be mentioned. The single endarch protoxylem group suggests a tendency which has been recorded in the petiole, but not the stem, of *Angiopteris*. On the other hand, there is no endodermis in the petiole of living Marattiaceae, and no sclerenchyma (except the hypodermal layer in *Angiopteris*). In the Psaroniae, however, which are generally considered to be related to the Marattiaceae, sclerenchyma is well developed, and Seward (40, p. 425) remarks on this point that it is a character of secondary importance which can readily be explained by differences in habit. Similarly the presence of a palisade pericycle, which does not seem to be found in the Marattiaceae, is not necessarily an indica-



tion of relationship to the Cyatheaceae, some fossil members of which possess a similar tissue.

Among known fossil ferns, the only one which even remotely approaches the arrangement in *Paradoxopteris* is the petiole described by Ogura as *Yezopteris polycycloides* from the Upper Cretaceous of Hokkaido. Here there are at least three rings of meristeles, with an arrangement which in parts suggests that of the bundles with accessory strands of *Paradoxopteris*. The tissues are not very well preserved, but a point worthy of notice is that in a section of Ogura's specimen B, presented by him to the British Museum (V. 20474), mucilage canals are present, more or less alternating with the bundles. There are also indications here and there of a pericycle composed of a regular layer of rectangular cells. Ogura suggested that although there were no definite grounds for referring this specimen to any particular group of ferns, it might possibly be a representative of a new family which would be ranked near the Cyatheaceae. *Paradoxopteris* and *Weichselia* have not so far been found higher than the lower Cenomanian, while *Yezopteris* was found in upper Cretaceous (Senonian) beds. An affinity, though not identity, with *Paradoxopteris* seems possible, and both ferns may either belong to the Marattiaceae or to an extinct allied group.

## VII. SUMMARY.

1. *Paradoxopteris* appears to be the rachis of the lower Cretaceous fern fronds long known under the name of *Weichselia*. Until proof of connexion is complete, the name *Paradoxopteris* may be retained, according to the usual palaeobotanical practice, for the structurally preserved specimens.

2. *Paradoxopteris*, a fern with very large fronds, has a highly dissected polycyclic stelar structure, with sometimes as many as eleven concentric circles of meristeles, alternating with circles of accessory strands. This structure cannot be exactly paralleled in any living or fossil fern.

3. *Angiopteris* offers the nearest analogy to *Paradoxopteris* in its stelar anatomy, and certain minor characters, including the presence of mucilage canals alternating with the meristeles, also suggest an affinity with the Marattiaceae. Neither the anatomy nor the nature of the supposed fertile fronds permits a final decision at present on the systematic position of this fern. It certainly does not belong to the Osmundaceae.

4. The tissue described by Hirmer as phloem is the pericycle, and usually consists of very large square or palisade cells. The occurrence of a rather large-celled pericycle in various recent ferns is noted, and attention is drawn to the palisade pericycle in *Protopteris cotleana*, *Protopteris arborescens*, *Fasciostelopteris tansleii*, and *Cyathorachis fujiana*.

5. The phloem is characterized by the large size of the sieve-tubes.

Tyloses sometimes occur in the tracheids. An endodermis is present, and the sclerenchymatous sheath is from 2 to 10 cells in width. The ground tissue contains secretory canals, alternating with and slightly external to the curved meristeles, and tannin cells. Similar canals were recorded by Schuster in a German *Weichselia* rachis, and help to confirm the connexion of *Paradoxopteris* with that genus.

6. Crystals of quartz in some of the cells, also recorded by Schuster in his *Weichselia*, were probably formed in the course of silicification and not as replacements of plant oxalates. On the other hand, silicious 'nodules' have been recorded in cells of some living Marattiaceae, but they seem to be confined to the epidermis.

7. The very complex stelar structure, the high proportion of sclerotic tissue, the development of a large-celled pericycle probably for water-storage, and the presence of mucilage canals all suggest a xerophytic habit, as do also the thick pinnules and the structure of the stomata.

8. The geologically slightly younger Japanese fern *Yezopteris polycycloides* may be related to *Paradoxopteris*.

9. The range of variation in *Weichselia* fronds is discussed in detail. No satisfactory basis for the specific subdivision of the genus has yet been brought forward. Lipps's attempt to recognize five different types (he does not attach names to them, only illustrates two, and does not clearly state the differences) cannot be considered until further detailed evidence is published.

10. *Weichselia reticulata* may none the less be a 'form species', including more than one natural species, the actual specific differences not being recognizable in isolated fragments of sterile fronds. As Berry has said (1928, p. 3): 'it certainly is anomalous to suppose that a single species ranges from the late Jurassic to the Upper Cretaceous' (Wealden to lower Cenomanian) 'and over at least five continents', though that is presumably not impossible.

11. *Weichselia erratica* Nathorst, *W. reticulata* var. *erratica* (Nath.) Schuster, and *W. peruviana* (Neumann) Zeiller cannot be distinguished from *W. reticulata* (Stokes and Webb). *Gleichenia*? *gilbert-thomsoni* Fontaine is also most probably the same species.

12. Certain fossils described by Stiehler as *Pandanus simildae*, by Trautschold as *Equisetites* sp. and *Calamites* sp., and by Schlagintweit as *Equisetites*? are most probably fragmentary impressions of the rachis of *Weichselia*.

13. A consideration of various records of supposed fertile fronds of *Weichselia* leads to a rejection of all except that first figured by Nathorst (but not recognized by him as belonging to *Weichselia*); that figured by Seward as *Conites minuta*, and subsequently discussed by Bommer; and similar fructifications recorded by Lipps. If these identifications are

confirmed, *Peruviophyllum minutifolium* Steinmann may also prove to be a fertile frond of *Weichselia*.

14. *Weichselia reticulata* is here recorded for the first time from the Albian of England.

15. Though characteristic of Wealden beds, it must be emphasized that *Weichselia* also occurs in the Aptian, Albian, and perhaps even Cenomanian. Caution is therefore necessary in assigning a geological age to any beds solely on the ground of the presence of this fern.

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## EXPLANATION OF PLATE XIV.

Illustrating Mr. W. N. Edwards's paper on 'The Cretaceous Fern *Paradoxopteris* and its Connexion with *Weichselia*.'

Figs. 1-8. *Paradoxopteris stromeri* Hirmer. Fig. 9 *Cyathorachis fujitana* Ogura. All transverse sections. Photographs by H. G. Herring and W. N. Edwards.

Fig. 1. A specimen from Baharia collected by Prof. Stromer (Munich). Nat. size.

Fig. 2. Part of the same. × 2.

Fig. 3. A single meristele from a Munich slide figured by Hirmer (1925, Pl. II, Fig. 4). *ph.* phloem; *per.* space formerly occupied by pericycle (cf. Text-fig. 2); *end.* endodermis. × c. 30.

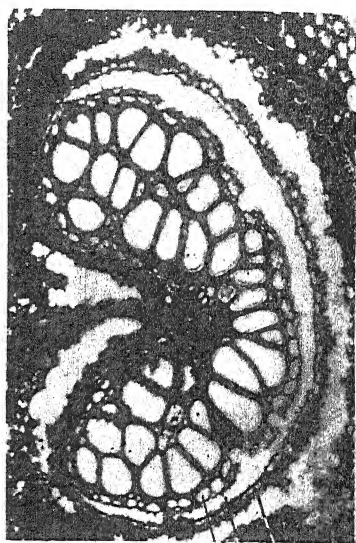
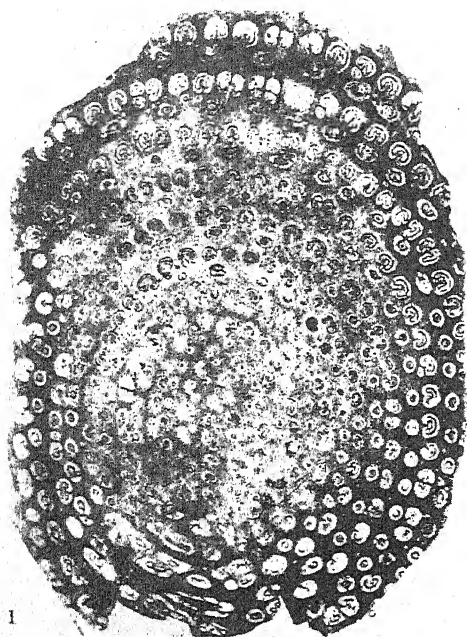
Fig. 4. Meristele almost surrounded by pericycle (Brit. Mus. Nat. Hist., V. 21368d). × c. 22.

Figs. 5 and 6. Accessory strands to show varying development of pericycle (V. 6124b). × c. 60.

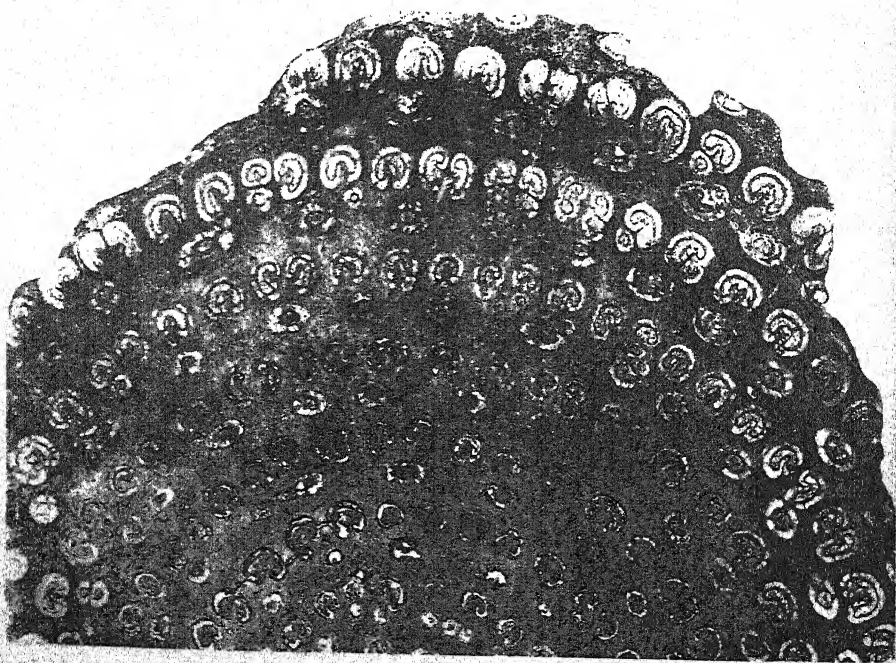
Fig. 7. Secretory canal (V. 6124d). × c. 60.

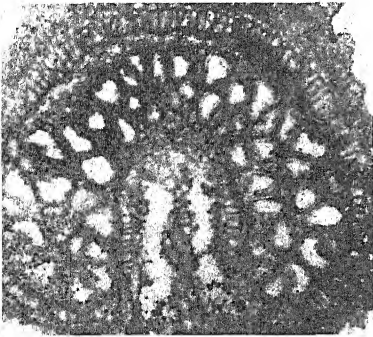
Fig. 8. Quartz crystals in xylem (Kidston unnumbered coll., slide 6). × c. 60.

Fig. 9. *Cyathorachis fujitana* Ogura. Upper Cretaceous, Yubari, Hokkaido, Japan; showing development of pericycle (V. 20458). × c. 60.

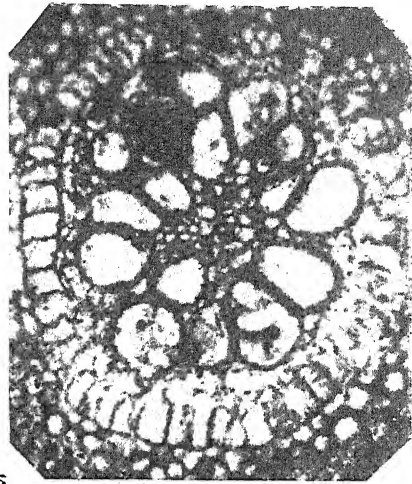


3  
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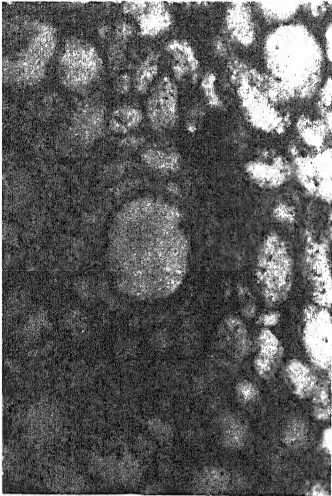




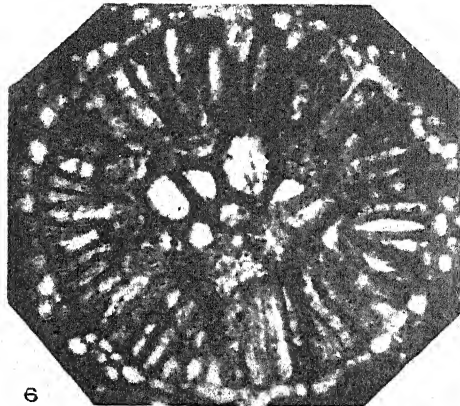
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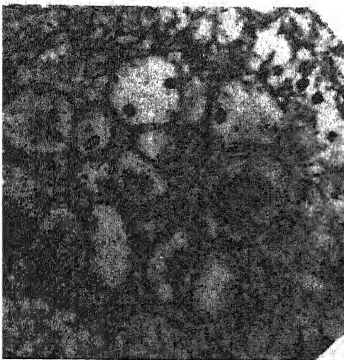
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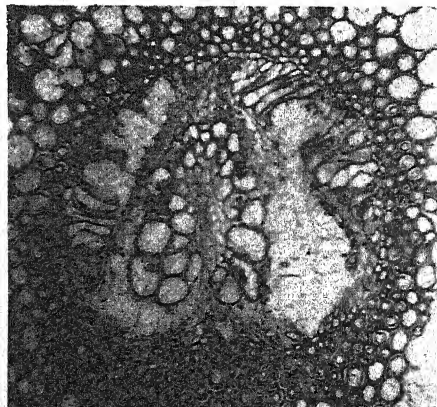
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8



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# Some Observations and Experiments on the Drought Resistance of *Pelvetia canaliculata*.

BY

W. E. ISAAC.

(Botany Department, University of Birmingham.)

With one Figure in the Text.

THIS work was carried out in July 1927 and in March and April of 1928 at Port Eynon on the coast of the Gower Peninsula, S. Wales.

The various species of Phaeophyceae growing within the littoral area have a definite and restricted range. Thus *Laminaria* is never found growing side by side with *Pelvetia*, neither is it found growing along with *Ascophyllum*. From this it follows that the various species of brown seaweed are exposed to the air for varying periods and thus probably vary in their capacity of resisting drought.

At Port Eynon the most abundant and prominent species of Phaeophyceae taken in order from low-tide mark upwards are: *Laminaria digitata*, *Fucus serratus*, *Ascophyllum nodosum*, *Fucus spiralis*, f. *platycarpus* Thur., and lastly *Pelvetia canaliculata*. There are, of course, other species of Phaeophyceae to be found, but they are mostly confined to rock pools, which are numerous and large. *Halidrys siliquosa*, *Cystoseira fibrosa*, and *Dictyota dichotoma* are among the permanently submerged algae of rock pools where there is a constant outflow of water.

Of all these plant inhabitants of the littoral area, the problem of drought resistance will be most important for *Pelvetia* since it extends highest up the beach and is consequently exposed to the air for the longest periods. In the lower parts of its zone at Port Eynon it forms fairly dense long masses on rock ledges which jut above the shallow sand deposit. In this region it is accompanied by *Fucus spiralis*, f. *platycarpus* Thur. The two species may be commingled or they may separate out into pure beds. In the higher parts of the zone, *P. canaliculata* alone of the brown seaweeds is found on the rock boulders, the plants occurring singly or in little groups. As would be expected, the *Pelvetias* growing highest on the shore are generally the most stunted, and the bare rock between them may be colonized by the lichen, *Verrucaria maura*. *P. canaliculata* is covered by

most, if not all, tides in the lower part of its zone. Also a certain amount of protection is afforded, (a) by aggregation of individuals, (b) by the fact that many of the plants lie upon the surface of the sand—which is more retentive of water than bare rock. The most highly situated plants are not covered at all by the neap tides, and many thus remain exposed continuously for a few days.

#### METHODS OF INVESTIGATION.

The whole plant of *Pelvetia* was weighed at intervals, and was kept in the meantime as near its natural position on the beach as possible.

Water loss in exposed marine algae is affected by many factors other than variation in tidal oscillation and in situation within the littoral area. The most important of these factors will naturally be the humidity of the air and thus measurements of humidity were made at the position of the exposed *Pelvetias*.

The humidity was measured as relative humidity by means of an Edney Hair Hygrometer (Pastorelli & Rapkin, Ltd.). The number of observations of relative humidity made during each experimental period is given in brackets after the average relative humidity (Table I).

#### EXPERIMENTAL.

Observation will make it clear that the situation of algae in relation to each other and the density of the algal covering at a particular spot is of significance in relation to water loss. The density in its turn depends upon the mass and absolute frequency (in Arrhenius' sense<sup>1</sup>) of the species. Isolated plants and those exposed at the surface of a bed of seaweed suffer most from the drying effects of physical conditions. The importance of mutual protection in decreasing the total loss in weight of seaweeds during their inter-tidal exposure is illustrated by the following experiment.

April 6, 1928. *Pelvetia*, Loss in weight, 8.75 hours.

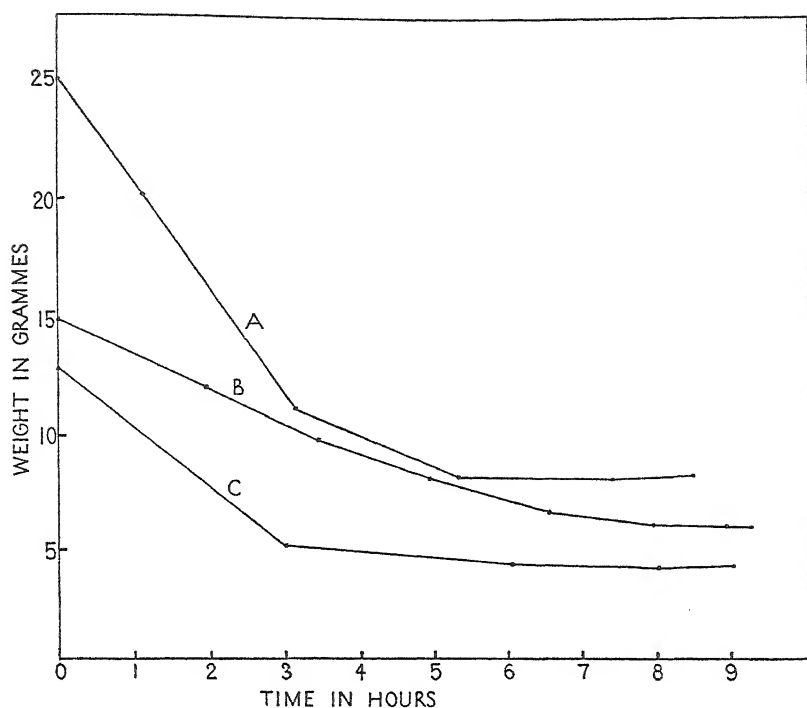
Exposed thallus: 60.39 per cent.

Thallus covered by others: 47.75 per cent.

Difference: 12.64 per cent.

The curves of mass against time obtained for *Pelvetia* during periods of exposure are typically hollow (Graph, p. 345). The results obtained are given in Table I below.

<sup>1</sup> Frequency estimations are usually indices of the frequency of a species of a plant community relative to the other species. Arrhenius in 1922 called attention to the possible importance in certain cases of *absolute* frequency, i.e. the actual number of individuals of a species present per unit of the plant community (1).



Loss in weight of *Pelvetia canaliculata* during inter-tidal exposure.

A. 25 March, 1928.  
B. 7 April, 1928.  
C. 18 July, 1927.

TABLE I.  
*Water loss in Pelvetia canaliculata.*

Date.	Average relative humidity.	Standard deviation of relative humidity.	Time.	Final loss in weight.
	%.		hrs.	%.
15. 7. 27	—	—	9.25	63.23
18. 7. 27	—	—	9	67.94
25. 3. 28	72 (9)	6.5	8.5	68.04
5. 4. 28	66.53 (18)	3.5	9	64.5
6. 4. 28	70.46 (20)	10.35	8.75	60.4
7. 4. 28	73 (14)	6.4	9.25	62.1

The loss in weight is remarkably high. The greatest loss takes place during the earlier part of a period of exposure and the plant tends to become air dry and hygroscopic, thus absorbing water vapour with any increase in the relative humidity of the air.

The air-dry condition is the commoner condition in the case of *Pelvetia* growing in the upper part of the zone—at least during the warmer

parts of the year. This would certainly be the case where isolated plants growing on a rocky substrate are exposed to the air continuously for a few days during neap tides. Further, the absolute frequency is so low in the upper reaches of the *Pelvetia* zone that any protection by 'mutuality' is rendered practically impossible. The degree of desiccation is indicated by the appearance and colour of *Pelvetia*. The air-dry plants are stiff, shrivelled, and dark brown (almost black) in colour.

Probably the majority of the plants found in the lower part of the zone do not become air dry regularly, although the loss in weight is very considerable. In the lower part of the *Pelvetia* zone, 'mutuality' may often be of importance.

The data given in Table I show no evident correlation between the amount of water loss and the relative humidity of the air. This can be accounted for by the fact that the relative humidity of the air may vary greatly throughout a period of inter-tidal exposure, and in addition we may get a rapid fluctuation in the humidity within a short period of time. In view of this, it is obvious that many readings of relative humidity should be made during any single investigation of water loss. The greater the number of readings taken the more accurate will be the calculated relative humidity. This principle, of course, holds good when the series of observations contains several identical readings. The value of the average humidity figures is indicated by the standard deviations included in the table as well as by the number of observations made. Really satisfactory results can only be obtained by means of a continuously recording instrument at the level of the plants studied.

Two comparisons were made of the water loss in *P. canaliculata* and *Fucus spiralis* f. *platycarpus*,<sup>1</sup> under the same conditions and using the same methods. The results are given in Table II below.

TABLE II.

*Comparison of Water loss in P. canaliculata and Fucus spiralis*  
f. *platycarpus*.

Date.	<i>P. canaliculata</i> . %.	<i>F. spiralis</i> . %.
5.4.28	64.5	65.85
6.4.28	60.4	63.5

From Table II it will be seen that *Fucus spiralis* f. *platycarpus* shows the same order of water loss as *Pelvetia*, but in the cases recorded its water

<sup>1</sup> See Börgesen (2). On exposure to air elongated bladders appear on either side of the midrib. These 'bladders' are not permanent structures such as are found in *F. vesiculosus*, but are temporary in character.

loss is slightly greater. The curve of weight against time (i.e. the nature of drying) is also of the same character as that of *Pelvetia*.

*F. spiralis* f. *platycarpus*, however, is covered by most, if not all, tides at Port Eynon, and the writer did not observe it growing elsewhere along the Gower coast; in this respect it presents a contrast to *Pelvetia*.

The power possessed by *P. canaliculata* of surviving the air-dry condition during long exposure under the influence of conditions which favour evaporation to a marked degree obviously fits it for growing in the upper limits of the littoral region; or, if the view is taken that it is excluded by competition from the lower zones of the seashore, it is clear that the power above referred to enables it to obtain a footing in a rather inhospitable habitat. A benthic organism to succeed under a given set of conditions must, however, be able to reproduce itself under those conditions. This *P. canaliculata* is able to do. As in other Fucaceae, the oogonia and antheridia are situated within conceptacles and the contraction of the thallus on drying is necessary in order to force out the sperms and oospheres. In addition to this, the two oospheres in *P. canaliculata*<sup>1</sup> are not liberated from the thick-walled mucilaginous oogonium, which the sperms have to penetrate before fertilization can take place. This, together with a physiological organization able to withstand the air-dry condition successfully, accounts for the fact that *Pelvetia* can flourish under such severe conditions, and failure in regard to one or both of these characters may account for the absence of other Phaeophyceae from the upper part of the *Pelvetia* zone.

*Pelvetia* recalls to mind such sclerophyllous xerophytes as the creosote bush (*Covillea glutinosa*) and *Myrothamnus flabellifolia*, which are both xerophytes of the 'drought-enduring' type. The creosote bush, which is found in the hottest and driest parts of the south-western desert region of North America, is able to withstand the drying of the soil to a depth of four feet to a level about 4.5 per cent.<sup>2</sup> below the wilting coefficient (5). In the case of *Myrothamnus*, which grows in cracks on granite slopes in Rhodesia, Thoday (6) was able to demonstrate a loss of water to the extent of 93 per cent. No such precise data are available in the case of the creosote bush, but Maximov, who visited the Colorado desert of Southern California in the autumn of 1926, expressed the view that 'during the dry season of the year, the leaves of the creosote bush and similar desert plants contain no free water whatever, all the water remaining in them being

<sup>1</sup> According to Moor (4), *Pelvetia fastigata* sometimes produces four functional oospheres per oogonium instead of the usual two characteristic of the genus. This species of *Pelvetia* is found on the west coast of North America from Oregon (Coos Bay) to the west coast of lower California, growing at a somewhat higher average level on the shore than does *P. canaliculata* on the shores of Britain.

<sup>2</sup> This figure was worked out from the data in Shantz and Piemeisel's paper (5).

firmlly retained by the cell colloids' (3). Probably this could also be said of *P. canaliculata* in many cases.

In conclusion, I should like to express my thanks to Professor R. C. McLean for the help he has given me during the course of this work.

#### SUMMARY.

1. *Pelvetia canaliculata* is exposed to the air for by far the greater part of the tidal period. In the upper part of its zone it may be exposed to the air continuously for several days during neap tides.

2. Losses in weight of 60 to 68 per cent. were recorded during periods of 8.5 to 9.25 hours.

3. The loss in weight was very marked at the beginning of a period of exposure and the plant tended to become air dry. During the greater part of the period of exposure there was but little change in weight.

4. Where in the lower part of the *Pelvetia* zone the absolute frequency is sufficiently high for the massing of individuals to result in overlapping, many *Pelvetias* will be protected and thus suffer less water loss. In the upper part of the zone the absolute frequency is very low, and thus there is no diminution of water loss due to mutual protection.

5. The lower part of the *Pelvetia* zone at Port Eynon is shared with *Fucus spiralis* f. *platycarpus* Thur. The latter shows the same order of water loss as *P. canaliculata*, but is covered by most, if not all, tides.

6. It would seem that *P. canaliculata* is able to grow in the upper limits of the littoral region as a result of the retention of its two oospheres within a thick-walled mucilaginous oogonium, together with the possession of a physiological constitution able to survive successfully the dry air condition.

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# Sexual Reproduction in *Macrocystis pyrifera* Ag.

BY

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With nine Figures in the Text.

IN 1926 a note on reproduction in *Macrocystis pyrifera* Ag. was published in this journal by Dr. E. M. Delf and the writer.<sup>1</sup> Up to this year pressure of work has prevented the continuation of the investigation, but early in June a plentiful supply of material was discovered among rocks on Woodstock beach in Table Bay. The accessibility of the spot and the abundance of fertile fronds on the plants growing there led to a fresh attempt being made to study the development of the gametophytes.

In the previous note it was stated that fertile fronds had been found from October to April. The presence of such fronds in June, now established, suggests either that the purely vegetative condition is of very short duration or that the vegetative period is not determined by a seasonal factor. The latter hypothesis seems the more probable, as in making collections of the seaweed some plants are always to be found without any sori on their fronds.

The material was collected at 12 noon on June 8 and brought dry to the University Laboratories. At 1 p.m. cultures were started. In each case a small piece of fertile frond was placed in 100 c.c. of sea water in a covered glass dish with a few cover-slips on the bottom. An hour later the pieces of frond were removed, and each culture was found to contain numerous actively swimming zoospores. The zoospores, although showing slight variations, were not of two distinct sizes.

On June 9 all but a few of the zoospores had settled down, and most had started to germinate. Fig. 1 was drawn with a camera lucida on June 10, and shows short germination tubes into which most, but not all, of the cytoplasm had passed. During the next week the young gametophytes seemed to make little progress, but after that development took place rapidly, and by June 25 mature gametophytes were present in all the cultures, while in one, several young sporophytes had been formed. Figs. 2-9

<sup>1</sup> In that note the name of the writer was spelt Levyn instead of Levyns.

are camera lucida drawings made from living material at this time. The cultures were not disturbed in any way. In previous attempts to germinate zoospores, weekly changes of sea water had been made, but as few of these

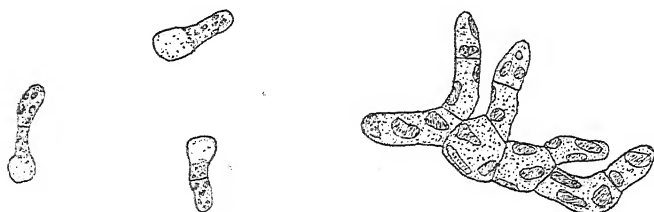


FIG. 1.

FIG. 2.

FIG. 1. Germinating zoospores, 2 days old.  $\times 800$ .

FIG. 2. Gametophyte, 17 days old.  $\times 800$ .

cultures produced results, on this occasion the dishes were left unattended. The vigorous growth of gametophytes that resulted seems to indicate that frequent changes of water are undesirable.

A well-developed gametophyte in which no antheridia or oogonia had been organized is shown in Fig. 2. Sterile filaments of this nature were fairly common, but as the period during which sexual organs are being produced extends over a fortnight at least, such filaments are probably ones that have not yet reached maturity. Fig. 3 is a filament bearing antheridia, one of which had discharged its spermatozoid. The antheridia may be distinguished from the vegetative cells by their pale coloration and relatively homogeneous nature of their cell contents. Figs. 4 and 5 show other forms of the male gametophyte. As all types may be found in the same culture, the cause of these differences in size and form is not apparent.

The actual escape of the spermatozoid from the antheridium was not observed, but the antheridia are so similar to those described and figured by Sauvageau (2 and 3), and others in *Laminaria* and *Saccorhiza*, that there can be little doubt of the correctness of the interpretation of these structures in this case. In cultures with numerous empty antheridia, spermatozoids were found swimming about. One of these is shown in Fig. 6, in which the unequal lengths of the two cilia are clearly seen.

The female gametophyte is smaller than the male, in some cases, as in Fig. 7, being reduced to a single oogonium. Even if the gametophyte consists of several cells, branching is not frequent as it is in the male. Any cell may become an oogonium, and is easily distinguished from purely vegetative cells by its larger size and pear-shaped outline. This is in accordance with observations made on other *Laminariaceae* (3 and 4).

The writer was fortunate in seeing the fusion of male and female nuclei during the process of fertilization. Several oogonia were noticed to which a pale, rather homogeneous body was attached at the narrow end.



In one case (Fig. 7 *a*) this body had obviously fused with the oogonium and two nuclei, side by side, were clearly seen within the oogonium. This oogonium was kept under observation, and after an hour and three-quarters fusion of the nuclei had taken place (Fig. 7 *b*).

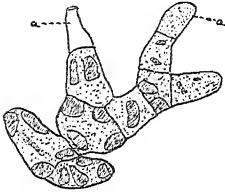


FIG. 3.



FIG. 4.



FIG. 5.



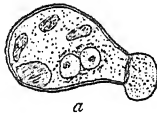
FIG. 6.

FIG. 3. Male gametophyte with two antheridia (*a*) from one of which the spermatozoid has escaped.  $\times 800$ .

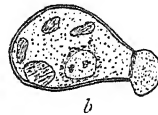
FIG. 4. Gametophyte with antheridia.  $\times 800$ .

FIG. 5. A small gametophyte bearing antheridia.  $\times 800$ .

FIG. 6. Spermatozoid.  $\times 800$ .



*a*



*b*

FIG. 7. Oogonium showing fertilization. (*a*) male and female nuclei associating; (*b*) male and female nuclei fused.  $\times 800$ .



FIG. 8.

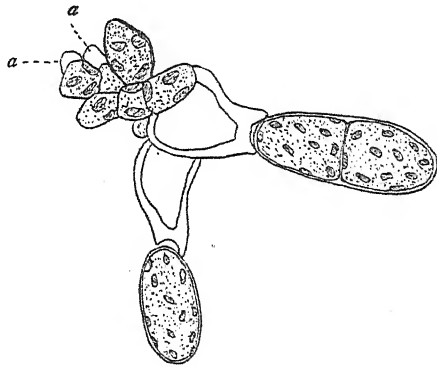


FIG. 9.

FIG. 8. Zygote escaping from the oogonium.  $\times 800$ .

FIG. 9. Gametophyte with two old oogonia from which the young sporophytes have been extruded. *a* = empty antheridium.  $\times 800$ .

Soon after fertilization the zygote assumes a wall of its own and the oogonium wall begins to swell. This swelling seems to force the zygote out of the oogonium through the weakened area in the wall at which point the spermatozoid was attached. Fig. 8 shows the extrusion of the zygote,

the remains of the spermatozoid being clearly seen to one side. After the zygote has been expelled the opening through which it has passed is obscured. In cultures the young sporophyte develops just outside the empty oogonium (Fig. 9), but under natural conditions it would almost certainly be dispersed by movements of the waves, as it is quite free from the parent gametophyte.

Before comparing fertilization and subsequent events in *M. pyrifera* and related forms, it is desirable to draw attention to the advantages to be derived from a use of methylene blue. Young gametophytes are hardly affected by this stain, but the greater part of the antheridial wall and an area at the narrow end of the oogonium stain a brilliant blue. Young sporophytes show a great affinity for this stain, giving a violet coloration. Williams (4) has drawn attention to the differential staining of gametophyte and sporophyte with this stain in *Laminaria*.

The male and female gametophytes in *M. pyrifera* agree closely with those described by other writers for *Laminaria*. There is no clear evidence to show that bisexual gametophytes occur. Cases such as that depicted in Fig. 9, where oogonia and antheridia appear to be associated, are probably due to two or more zoospores having been grouped together before germination.

The chief difference between the present example and other members of the Laminariaceae where similar details have been studied, is to be found in the time at which fertilization occurs. In *Laminaria* both Sauvageau (3) and Williams (4) have shown that the egg is extruded before fertilization, the second-named author providing conclusive proof of this, in that he was able to follow the process of nuclear fusion. In *Chorda*, Williams (4) finds that the egg grows out of the oogonium still enclosed in the extensible inner wall of the oogonium. Fertilization has not been observed in this genus. In *Macrocystis* the egg is fertilized within the oogonium, and the process of extrusion takes place *after* the zygote has been formed. The method by which extrusion takes place appears to be the same, whether the body extruded be an egg or a zygote. Subsequent development agrees closely in *Laminaria* and *Macrocystis*.

#### SUMMARY.

1. Plants of *M. pyrifera* have been found in the fertile condition from October to June.
2. Cultures started in June showed germination of many of the zoospores after twenty-four hours.
3. Mature gametophytes, similar to those in *Laminaria*, were present in cultures seventeen days old, and in one case young sporophytes were present.

4. Antheridia and oogonia agree closely with those of *Laminaria*.

5. Fertilization occurs within the oogonium and the zygote is extruded in the same manner as the egg in the other genera that have been investigated.

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2. SAUVAGEAU, C.: Comptes Rendus de l'Académie des Sciences, clxi. 796, 1915.
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4. WILLIAMS, J.: The Gametophytes and Fertilization in *Laminaria* and *Chorda*. Ann. Bot., xxxv. 603, 1921.



# A Note on the Structure of the Phyllodes of *Oxalis* *Herrerae* R. Knuth and *O. bupleurifolia* St. Hil.

BY

C. R. METCALFE.

(Assistant Keeper of Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey.)

With seven Figures in the Text.

THE Director of the Royal Botanic Gardens has handed over to me the material of *Oxalis Herrerae*, which he collected in Peru, for investigation and comparison with *O. bupleurifolia*, and has supplied the following note about this interesting species :

‘When travelling in the Andes of Peru in March 1903, I found near Ollantaytambo, between Urubamba and Cuzco, a remarkable *Oxalis* with fleshy petioles, cactoid in character and, when growing in exposed stony situations, devoid of the characteristic three leaflets. In shady positions the petioles were more or less normal, though slightly thickened, and they bore three large green leaflets. In dry situations the leaflets were small, reddish in colour, dependent, and with no power of movement. Frequently they were aborted, and the fleshy fusiform petioles or phyllodes were the sole assimilating organs (Fig. 1, *a-d*). The flowers, which are yellow, are trimorphic.

‘It was recognized that this was an undescribed species, but unfortunately the material, both dried and in spirit, which was brought home, was not fully worked out at the time, and the species, which was again found in the Urubamba valley by Herrera in 1928, has been described by Knuth under the name *Oxalis Herrerae* in ‘Engl. Pflanzenreich, Oxalid. (1930), p. 115 . . . A. W. Hill.’”

*O. bupleurifolia*, St. Hil., which has long been in cultivation at Kew, is of interest in comparison with *O. Herrerae*, since the petiole has taken on the functions of the leaflets and has become the assimilating organ, but in this case it has developed as a flattened leathery phyllode, closely resembling the phyllodes of the Australian acacias, and in particular those of *Acacia armata* R. Br. As with *Acacia*, the leaflets are fully developed

in young plants, but later they tend to be undeveloped and the plant is provided with the flattened phyllodes only.

Very little anatomical work appears to have been done on the genus, but Solereder<sup>1</sup> refers to *O. carnososa* Moll., and other species in which the

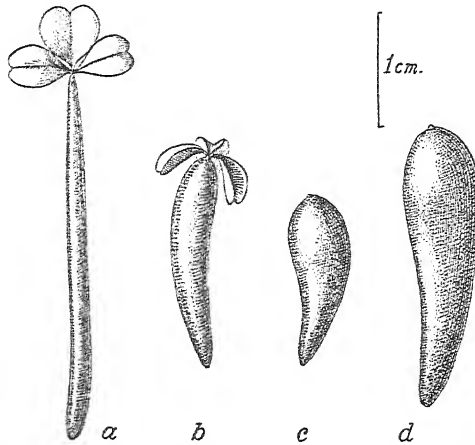


FIG. 1. *a*. A normal leaf of *Oxalis Herrerae*. *b*. A leaf with small leaflets and swollen petiole. *c* and *d*. Swollen petioles from which leaflets have fallen.  
(Figure by G. Atkinson.)

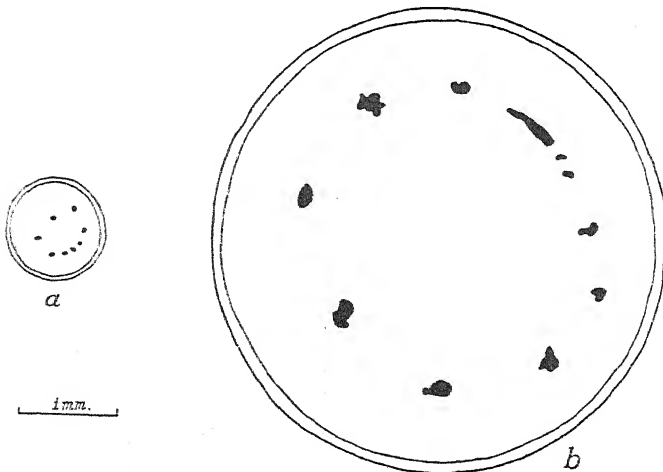


FIG. 2. *a*. Transverse section through middle of an unswollen petiole of *O. Herrerae*.  
*b*. Transverse section of a swollen petiole.

cells of the epidermis of the leaf, especially on the upper surface, are large, and serve for the storage of water. In the petiole of *O. tetraphylla* Cav. the vascular system is of some interest, since in the lower portion there are nine isolated bundles, while in the upper part of the same petiole the

<sup>1</sup> Systematic Anatomy of the Dicotyledons. Oxford, 1908, 170 and 851.

xylem and phloem form a continuous ring, within which there are two medullary bundles. In *O. latifolia* H.B.K., on the other hand, there is a single ring of bundles (and no medullary bundles) throughout the length of the petiole.

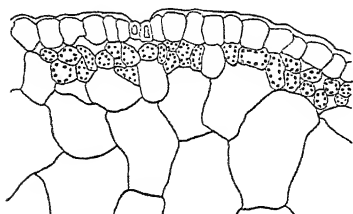


FIG. 3.

FIG. 3. Transverse section showing peripheral tissues in an unswollen petiole of *O. Herrerae*.

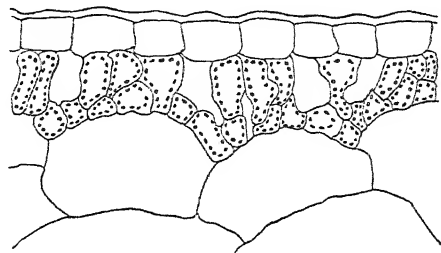


FIG. 4.

FIG. 4. Peripheral tissues of a swollen petiole of *O. Herrerae*.

The vascular system of the petiole of *O. Herrerae* is found to consist of a ring of separate bundles similar to that described for *O. latifolia*. The diameter of a petiole or phyllode of *O. Herrerae* may increase from 1 mm., with leaflets attached, to 4.5 mm. after the leaflets have fallen, and the arrangement of the bundles is shown somewhat diagrammatically in Fig. 2, *a* and *b*, which represent transverse sections through the middle of two such petioles drawn to the same scale. The remarkable difference in the diameters of the petioles is due chiefly to an extension of the bulk of the parenchymatous tissues of which the petioles are chiefly composed. Beneath the epidermis there is a layer of chlorophyll-containing cells, one or two cells deep, the inner limit of which is indicated by the inner circle shown in Fig. 2, *a* and *b*. The epidermis of an unswollen petiole (Fig. 3) consists of fairly large cells with an external covering of cuticle, interrupted at intervals by stomata. Beneath the epidermis comes the layer of cells containing chlorophyll. These are not arranged in a definite palisade, but are irregularly distributed, fairly tightly packed, with here and there intercellular spaces. At intervals among the assimilating cells there are single, large, parenchyma cells devoid of chlorophyll. The ground tissue of the petiole is composed of large, thin-walled parenchymatous tissue.

Certain changes take place in the structure of the swollen petioles or phyllodes when they become the principal photosynthetic organs (Fig. 4). The chief of these are (1) the increase in size of the epidermal cells and the thickening of the cuticle; (2) the increase in size of the chlorenchyma cells, the outermost of which become arranged in a roughly palisade manner; (3) the greater development of the intercellular spaces among the

assimilating cells; (4) the increase in size of the ground parenchyma cells compared with those in the petioles with leaflets still attached; (5) the greater frequency of stomata in the epidermis (Fig. 5); and (6) the absence of any cells devoid of chlorophyll among those containing chlorophyll, which are seen in the petioles of plants growing in damper places.

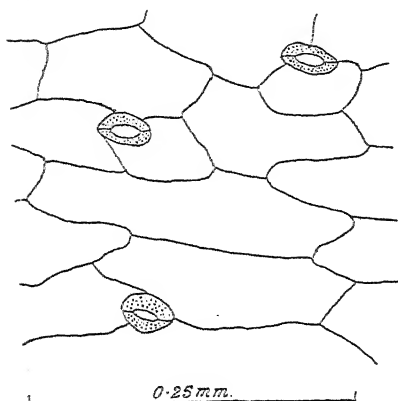


FIG. 5.

FIG. 5. Epidermal cells and three stomata from a swollen petiole of *O. Herrerae*.

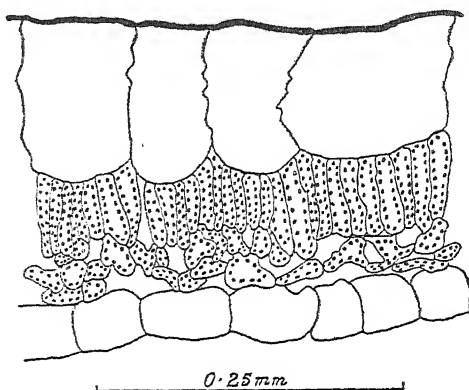


FIG. 6.

FIG. 6. Transverse section of leaflet of *O. Herrerae*.

The cells of the upper epidermis of the leaf of *O. Herrerae* (Fig. 6) are remarkably large, as is the case in *O. carnosa* and other species. In *O. Herrerae* they occupy more than one-third of the total width of the leaflet, whilst the cells of the lower epidermis are also relatively large. Stomata in the leaflets are confined to the lower surface. In some instances the leaflets do not grow more than a few millimetres long before they fall off, and in such cases the upper epidermal cells occupy an even larger proportion of the thickness of the lamina than do those in the larger leaflets of plants growing in damper places; the small leaflets thus tend to be slightly succulent, and show a corresponding modification to the fleshy petioles. The cuticle on both surfaces of the small leaflets is also thicker than on the larger ones. This is especially noticeable on the lower surface.

The structure of the axis shows no features of particular interest. A phellogen arises in the hypodermis at an early stage and gives rise to cork cells on the outside. The cortex is composed of cells of very varying sizes, of which the larger ones are mostly devoid of contents, whereas the smaller ones contain numerous small starch grains, and sometimes a single large crystal. A single ring of separate bundles is present in very young stems, but later on a cylinder of xylem is produced by the activity of a cambium. The rather infrequent vessels have scalariform thickening



and simple perforations. The pith consists of parenchyma cells containing a little starch and occasionally large crystals.

*O. bupleurifolia* also possesses deciduous leaflets in the same way as *O. Herrerae*, but the structure of the petioles of these two species differs markedly. In *O. bupleurifolia* the petiole is flattened and is a typical

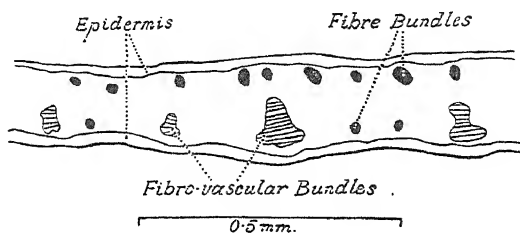


FIG. 7. Diagram of transverse section of part of a petiole of *O. bupleurifolia*.

phyllode and presumably always functions as the chief photosynthetic organ, especially after the leaflets have fallen. In spite of its laminate nature the petiole retains some evidence of its original radial symmetry (Fig. 7). On both surfaces there is a well-defined epidermis, perforated by stomata. The internal ground tissue consists of rather loosely arranged parenchyma cells containing chloroplasts. An interesting feature is the arrangement of the bundles, which are situated around the periphery of the phyllode. Towards the abaxial side most of them are large, consisting, in addition to fibres, of xylem towards the upper and phloem towards the lower surfaces, but among them there are also a few smaller ones which are chiefly composed of fibres. On the adaxial side, however, the bundles are smaller and consist almost, if not entirely, of fibres. It seems possible that these adaxial fibre bundles may represent reduced fibro-vascular ones in an originally radially symmetrical petiole.

The epidermal cells of the leaflets of *O. bupleurifolia*, as in other species, are very large in proportion to the total thickness of the lamina.



# Archaeopitys Eastmanii

BY

D. H. SCOTT, F.R.S.

With Plate XV and five Figures in the Text.

THE genus *Archaeopitys* was founded in 1914, in a paper by Professor E. C. Jeffrey and the present writer 'On Fossil Plants showing Structure from the Base of the Waverley Shale of Kentucky' (6) p. 345. The plant is thus of Lower Carboniferous age. The specimen then described showed no outgoing leaf-traces and hence there were no sufficient data for determining the upward and downward directions in the fragment. The conjectural determination then made has unfortunately turned out to be wrong, as I have already stated elsewhere (5) p. 261.<sup>1</sup> It is one object of the present communication to describe the true course of the vascular strands, as shown in a further specimen, which has since come under observation.

The second specimen was derived from the same deposit as the original one. It was received, with other fossils, from Professor Jeffrey, the author's collaborator in the memoir of 1914. Dr. Moritz Fischer was again the collector. The second specimen shows the exit of several leaf-traces through the wood, and thus leaves no doubt as to which is the top and which the bottom of the fragment. Consequently the course of the xylem strands can be followed accurately, and the information thus obtained, enables us to interpret correctly the original specimen also, which was not the case before.

The specimen now to be described shows more than half the transverse section of the stem (Pl. XV, Fig. 1). The maximum diameter is about 4.5 cm. and that of the pith about 3 cm. Nothing is present outside the secondary wood, nor can we be certain that the latter is complete. The middle part of the pith is in a decayed condition, but a thickness of quite a centimetre from the inner edge of the wood is preserved in places. Thus the dimensions are far larger than in the original specimen, which did not exceed 2.7 cm. in total diameter, that of the pith being only 5.5 mm. The

<sup>1</sup> It is fair to add that I was solely responsible for this error. My colleague was not concerned in this part of the memoir.

latter dimension only is significant, for the outer limits of both specimens are probably accidental.

A series of 20 transverse sections was cut by Mr. Hemingway. He also prepared a longitudinal series of 8 sections.

#### GENERAL STRUCTURE.

The pith consists of large short cells, as in the former specimen. The xylem-strands may be distinguished, as before, into circum-medullary and medullary, the former being those which form a ring immediately within the wood (Pl. XV, Figs. 1, 2). In the more complete sections, 16 circum-medullary strands were counted in a part corresponding probably to rather more than half the circumference of the pith. Thus the total circum-medullary set may have numbered about 30 strands. Not many of these are actually in contact with the wood—usually a few pith-cells intervene. Where there is direct contact it is commonly at points where the strand is about to pass out as a leaf trace.

The medullary strands extend all through the part of the pith preserved, up to a distance of 1 cm. from the inner border of the wood. They are sparsely scattered, especially in the deeper regions of the pith, where two neighbouring strands may be as much as 3 mm. apart. It is impossible to decide whether the strands extended to the centre of the pith—probably a few may have done so.

While the circum-medullary strands are fairly large (Text-figs. 1, 2, and 4) the medullary are smaller, especially those which are deeply seated in the pith. These inner strands often show only half-a-dozen tracheides (sometimes as few as 4) in transverse section.

In all cases the smallest elements lie at or near the middle of the xylem-strand, which may thus be called mesarch.

#### COURSE OF THE LEAF-TRACES. (NEW SPECIMEN).

Four leaf-traces are shown in the specimen, and three of these can be followed far enough to show them passing out through the wood. The direction in which they pass out is obviously the upward direction, and thus the top and bottom of the specimen are determined. The series runs from below upwards, slides 425 to 444.<sup>1</sup>

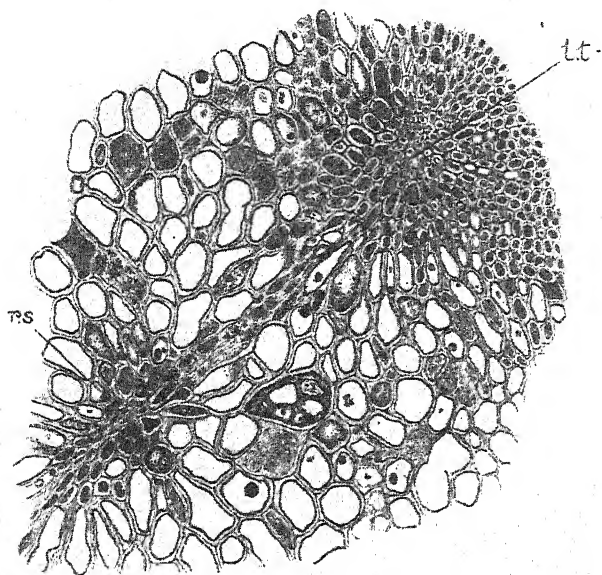
The trace which can be followed furthest is first clearly seen in 426 and extends through 18 sections to 443. The changes in its course are as follows:

In the lowest section (426) a large mesarch strand is seen just inside a wedge of the secondary wood, and almost if not quite in contact with it. The centrifugal primary tracheides of the strand are elongated radially,

<sup>1</sup> These numbers refer to slides in the Scott *Private* Collection.

and contrast sharply with the isodiametric secondary tracheides. So far there is nothing to show that the strand is about to make its exit as a leaf-trace.

In the next section (slide 427) the strand is much stretched radially and is becoming double, tending to divide into an inner and outer portion.



TEXT-FIG. 1. Separation of leaf-trace from reparatory strand *lt.*, leaf-trace, adjoining the secondary wood. *r.s.* reparatory strand entering pith, but still connected with the trace.  $\times$  about 40. Slide 431 (G.T.G.).

Two sections above (429) the two strands are separating, and their protoxylem-groups are widely apart. The inner (reparatory) strand is much smaller than the outer (the leaf-trace). There is much radial elongation of the tracheides as the strands separate. In the next section (430) the strands still appear to be connected by tracheides, but in 431 (Text-fig. 1) they have practically separated, though it is possible that some of the intervening elements may still be tracheal.

Two sections higher (433) separation is complete. The two strands are here nearly 2 mm. apart, centre to centre. The outer strand, in close contact with the wood, is typically mesarch, almost circular in outline, and about  $600\mu$  in diameter. The distinction between the primary and secondary centrifugal elements is fairly well marked, though there is a tendency to radial arrangement in the former also. The inner strand is much elongated radially, its exact limits are difficult to fix, but its radial diameter appears quite equal to that of the leaf-trace, while its tangential measurement is only about half as great ( $300\mu$ ). The inner (reparatory)

strand does not lie directly behind the leaf-trace, but a little to one side, and its long axis is in the same oblique direction.

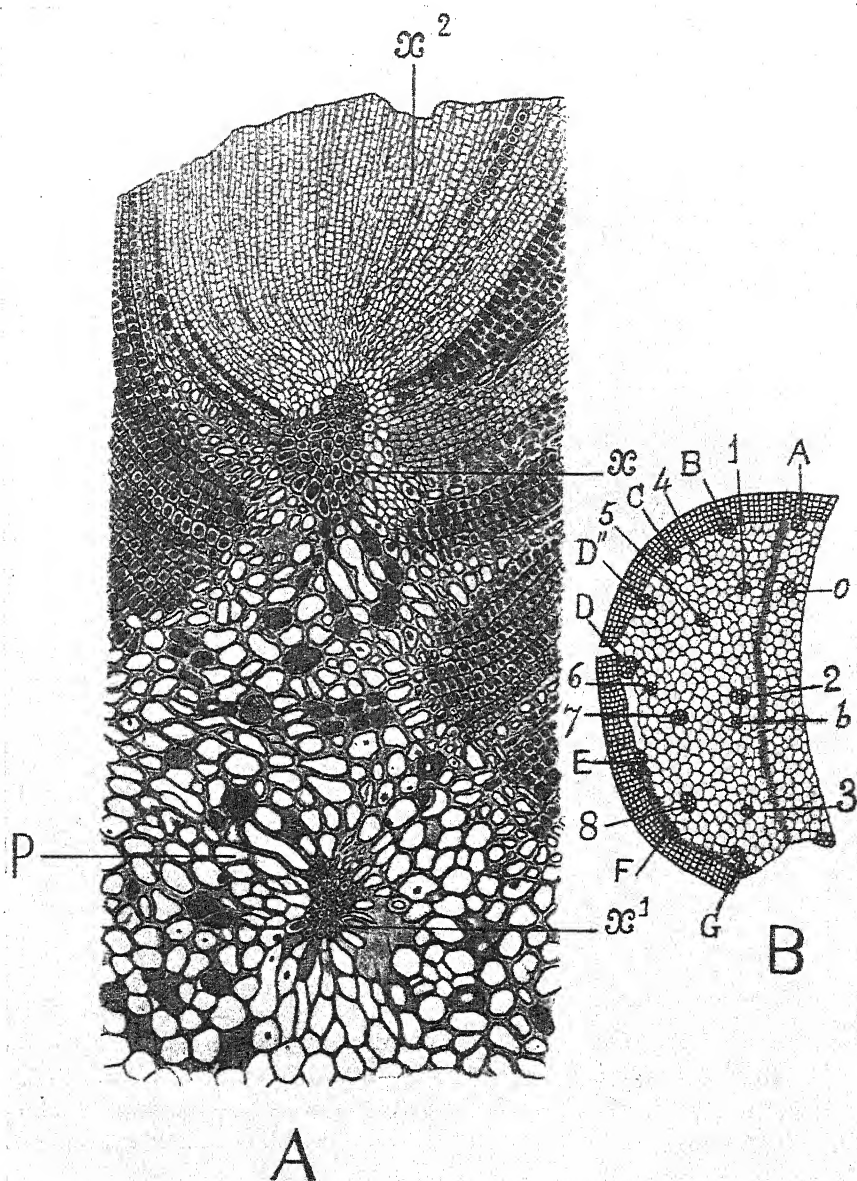
In the succeeding sections the strands continue to more further apart, the reparatory one swinging round towards the wood. The leaf-trace becomes more embedded in the wood and soon a bay begins to form behind it. In section 436 the bay is well marked, and the exit of the leaf-trace has definitely begun. A stage when the bay subtending the leaf-trace is deep, and the reparatory strand lies well back, is shown, from another leaf-trace in Fig. 2, A.

Returning to the former series, we find that in the section 438, the leaf-trace shows signs of a second division, giving off a small strand on the inner side. In the next section (439) this division is complete, the small intermediate bundle thus given off lying behind the leaf-trace at a distance from it of about 1 mm., while the original reparatory strand is about 2 mm. further off, and has moved considerably more towards the neighbouring secondary wood.

Two sections above (441) the leaf-trace is almost half-way through the zone of wood, and is acquiring a secondary wood-zone of its own. The intermediate strand can still be recognized—the reparatory strand is as before.

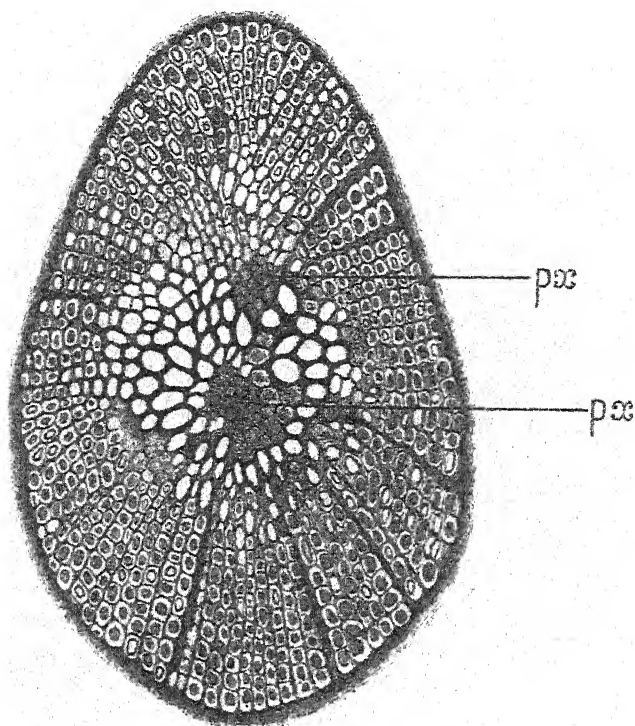
In the succeeding section (442) the secondary wood has closed in behind the outgoing trace. The latter is now completely surrounded by a secondary zone of its own, the whole thickened strand having an oval outline, with the long axis radial. The oval form is probably due to the oblique course of the strand. The intermediate strand is not to be seen, perhaps it may be lost in a damaged part of the tissue. The reparatory strand maintains about the same position as before.

In the last section showing this leaf-trace (443), it has nearly reached the outer border of the wood (as preserved). The oval outline of the trace is very clearly marked; the secondary zone is thicker on the abaxial than on the adaxial side (Text-fig. 3). In the primary xylem of the trace two internal small-celled groups (protoxylem) can be distinguished, lying one behind the other on the same radius. The concentric structure and other details of the leaf-trace are of interest for comparison with *Pitya Dayi*, Gordon. A group of apparent tracheides, lying close to the inner edge of the wood, and directly behind the leaf-trace, may probably belong to the intermediate strand. The reparatory strand is shown as before. It is here only about 1 mm. from the secondary wood. The giving off of a second or intermediate strand by the outgoing trace appears not to be a constant feature, though observed in one other leaf-trace. In a third leaf-trace, which was followed as far as possible, the protoxylem of the trace divides early, but the whole passes out and no intermediate strand is formed. The reparatory strand is evident.



TEXT-FIG 2. A. More advanced stage of separation, from another trace.  $\alpha$ , leaf-trace, entering wood.  $\alpha^1$ , reparatory strand, in pith.  $\alpha^2$ , secondary wood.  $p$ , pith.  $\times$  about 30. Slide 425 (G.T.G.). B. Diagrammatic transverse section from upper end of old specimen. 0-8 medullary strands. A-G circum-medullary strands.  $\delta$ , a small transitory medullary strand. For details see text.  $\times$  about 8. Scott Coll. 2909 (G.T.G.).

We have seen that some of the medullary strands are given off as reparatory strands from the outgoing leaf-traces. Others, however, arise from a division of circum-medullary strands which are not immediately

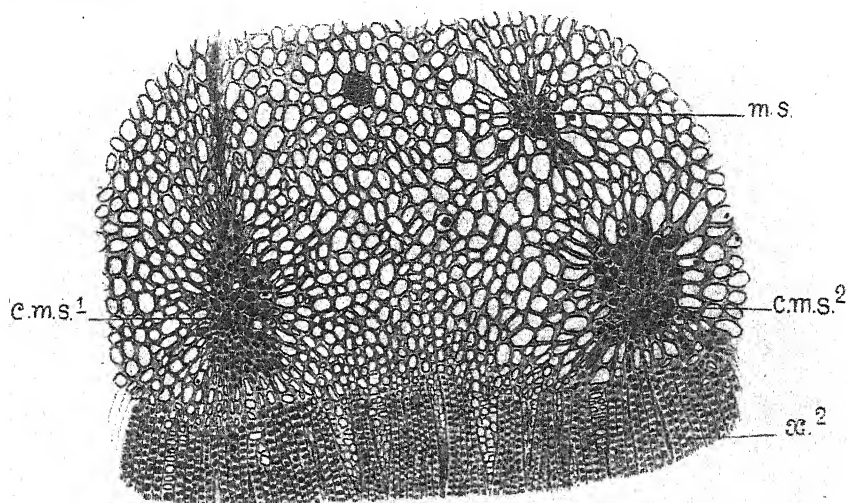


TEXT-FIG. 3. Leaf-trace far out in the wood, with a thick zone of its own secondary xylem. The narrow end points *inwards*. *px*, *px*, the two protoxylem-groups.  $\times$  about 45. Slide 443 (G.T.G.).

passing out. A number of cases of this kind have been observed. Two are represented in Text-fig. 4, from section 431. The circum-medullary strand marked *c.m.s.*<sup>1</sup> is about to divide; the division is completed in the next section above. That marked *c.m.s.*<sup>2</sup> has already some seven sections below given off the medullary strand, *m.s.* The separation is not quite complete in the first section of the series. Both the circum-medullary strands can be traced up to the eighth section above (332.15) and show no sign of passing out, or even coming into closer contact with the secondary wood; therefore they cannot be interpreted as departing leaf-traces whatever may become of them still further up. Other cases show the same thing—medullary given off from circum-medullary strands which do not pass out immediately.



It is of course possible that all the circum-medullary strands are really leaf-traces, in which case we must assume a complex phyllotaxis, and conclude that the leaf-trace gave off medullary strands at various successive



TEXT-FIG. 4. Circum-medullary strands giving off medullary strands. *c.m.s.*<sup>1</sup> circum-medullary strand preparing to divide. *c.m.s.*<sup>2</sup> another such strand, which had already given off the medullary strand, *m.s.* *x*<sup>2</sup>, secondary wood. *x* about 25. Slide 431 (G.T.G.).

points in its course. At any rate, the evidence shows that medullary strands are given off as branches, in the upward direction, from those at the periphery of the pith.

Occasionally the medullary strands themselves divide. Thus in the lowest section of the series (425) a deep-seated medullary strand is elongated in transverse section, and evidently preparing to divide. In the next section above division into two strands is complete.

Thus, as we follow the changes of structure in the upward direction, the number of medullary strands tends to increase, by the successive giving off of such strands from the circum-medullary bundles, and occasionally by further division of the medullary strands themselves. Yet the total number appears to remain fairly constant. What, then, becomes of the medullary strands in their upward course. Sometimes they are merely lost from view, passing towards the middle of the pith and then disappearing in the destruction of the central tissue. Sometimes, however, a medullary strand appears to come to an end while still in the well-preserved part of the pith. It is probable that such strands may re-unite with the parent circum-medullary bundle, or possibly may fuse with one another.

Our knowledge of the course of the strands remains imperfect. As we have just seen, the fate of the medullary strands is somewhat obscure; neither do we know how the outgoing leaf-trace is replaced. The series, long as it is, does not extend far enough to show what becomes of the reparatory strands. Probably each such strand takes up a circum-medullary position, and eventually becomes, or gives off, a new leaf-trace, but this is a matter of conjecture.

One thing, however, is clear. The exit of the leaf-traces gives the upward and downward directions in the stem, and we therefore know that the medullary are given off from the circum-medullary strands in the upward direction, the reverse of what was assumed in the case of the old specimen described in the Kentucky memoir. It will therefore be necessary to re-describe shortly the behaviour of the strands in that specimen, now that we can put it the right way up.

#### COURSE OF THE LEAF-TRACES. (THE OLD SPECIMEN).

In the Kentucky paper it was erroneously inferred that the outward movements of the medullary strands indicated that they were being followed in the upward direction<sup>1</sup> (6) p. 349. In the absence of outgoing leaf-traces the error may have been excusable, but as a matter of fact the true direction is the reverse of that assumed. The medullary strands are not leaf-traces, but rather of the nature of reparatory strands; as they separate from the circum-medullary bundles they pass upwards and *inwards*, into the pith.

That this is so is proved by comparison of the old with the new specimen. Thus in the Kentucky memoir the same two strands, one circum-medullary, the other medullary, are figured at two different levels, l.c. Pl. XXXVIII, Figs. 17 and 18. In the one they are just separating, in the other they are wide apart, the medullary strand having moved further into the pith. We now know, from comparison with stages in the new specimen (Text-figs. 1, 2, and 4) that the former stage (Text-fig. 18 of the Kentucky paper) is the lower and the latter (Text-fig. 17) the higher. The order of the whole series of transverse sections of the old specimen is the reverse of that assumed in the Kentucky paper. Text-fig. 4 (l.c. p. 348), is thus at the top of the series of 24 sections, while Text-fig. 5 (l.c. p. 349), represents a section near the lower extremity. Throughout the description *upper* must be substituted for *lower*, and *vice versa*.<sup>1</sup>

If we trace the old series in the same direction as the new, i.e. from below upwards, we find the same phenomena as those described above. The old specimen has in so far the advantage, that the cases of division of circum-medullary strands are more numerous. No less than 5 of the 7

<sup>1</sup> Alternate slides of this series are in the Scott Collection, numbered (in descending order) 2909-2920. The intermediate sections are in the possession of Prof. E. C. Jeffrey.

circum-medullary strands shown in the lowest section, give off medullary strands in the course of the series. This is already stated in the Kentucky paper, but there 'fusion' is spoken of, where, following the upward direction, we must now use the term 'division'. A couple of minor corrections have also to be made as the result of a re-examination of the old specimen.

Text-fig. 2, B, represents the uppermost section of the series.<sup>1</sup> The circum-medullary strand marked D 11 turns out to arise by the division of D, and not from that of C, as formerly believed. This division into two circum-medullary strands, is exceptional; in this case it takes place at a lower level than the normal division by which D gives off the medullary strand marked 6. In another instance the reverse is the case. The strand marked F, above where the medullary strand 8 is separated, divides again to form an extra circum-medullary strand on the side towards G, with which latter bundle it probably fuses at about the level shown in the diagram.

We may recapitulate the other changes in order. Fig. 2, B, will serve for illustration, but for details the Kentucky paper must be consulted. At the bottom of the series the strand E gives off the medullary strand 7. F next divides, giving rise to strand 8. In the same section G gives off medullary strand 3. C follows a little higher up, the resulting medullary strand 4 remains for some distance in contact with the secondary wood (on the side towards B) before it passes into the pith. In the meantime D divides to give off the small medullary strand 6, dividing again further up, as already mentioned, to form an extra circum-medullary strand.

The question arises, are any of these circum-medullary strands actual outgoing leaf-traces? There is no proof of their exit within the limits of the fragment, but some of them become partly embedded in the secondary wood (especially C, D, and E) and may be thus preparing to pass out.

The medullary strand 5 divides into two in the section (S. Coll. 2905) which comes just above that figured. This is the same phenomenon as we met with in the new specimen, so that in both stems, a medullary strand occasionally divides, as we trace it upwards, on its own account (6) Text-fig. 5, p. 349.

There is no doubt in this case that the number of medullary strands increases from below upwards. At the bottom of the series there are only 3 large medullary strands free in the pith. At the upper ends of the series (Text-fig. 2 B) in the corresponding part of the section there are 8 such strands, 5 having been given off from circum-medullary bundles as above described. This marked increase may indicate that the lowest section was cut near the base of a branch.

<sup>1</sup> One more section (S. Coll. 2905) though cut independently, comes immediately above the section figured (S. Coll. 2909).

There are also a few small and somewhat obscure medullary strands, the nature and fate of which remain doubtful. At the bottom of the series there are at least 4 such small strands in the pith. They all disappear as we follow the series upwards. They may actually die out and end blindly, as was supposed at the time the Kentucky Memoir was written.<sup>1</sup> The possibility of fusion with other medullary strands is not, however, excluded.

In the section shown in the diagram (Text-fig. 2, B) near the top of the series, one small medullary strand (*b*) is shown very distinctly; it first appears about 3 sections lower down, perhaps as an offshoot from the larger medullary strand no. 2. In the uppermost section of all it has disappeared again. Some of the smallest strands are still more transitory. There is no case known of the disappearance of one of the principal medullary strands; i.e. those which are given off from the circum-medullary bundles. The strand 6, however, given off from D, soon becomes unusually small (see Text-fig. 2, B).

Thus, in both specimens some uncertainty is left as to the course of the strands. We know that the medullary arise either from circum-medullary bundles (the commoner case), or by division of their own kind, but their ultimate fate, as followed upwards, whether they always re-fuse with other strands, or sometimes merely die out, remains undecided.

#### STRUCTURE OF THE XYLEM-STRANDS.

The new specimen adds nothing to our knowledge of this. In the longitudinal sections the pitting of the primary tracheides is seldom clear. Where visible it appears to be scalariform—no spiral elements are recognizable, though in one case a very narrow element (presumably protoxylem) is seen in the middle of a medullary strand. The strands, both circum-medullary and medullary are evidently mesarch as in the original specimen.

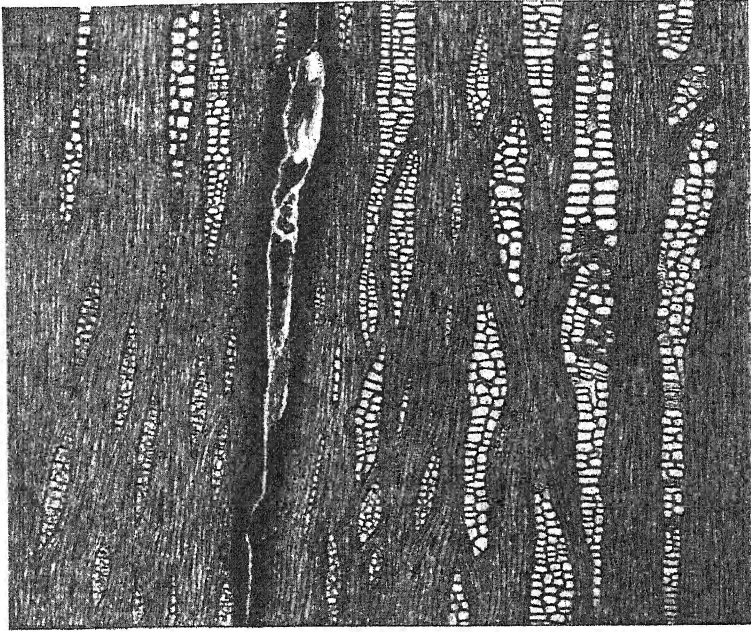
#### THE SECONDARY WOOD.

The secondary wood in the new specimen shows the same structure as in the original one (6) p. 351, with some slight differences due to the greater size of the stem. The largest medullary rays may be as much as 1.25 mm. in height, and 6 cells broad in the middle, though this is rare. A breadth of about 4 cells commonly occurs. Uniseriate rays are also common, from 2 to 9 cells in height (Pl. XV, Fig. 3, Text-fig. 5). Occasionally a single-celled ray is met with in tangential section.

One tangential section passes through the innermost part of the wood,

<sup>1</sup> I.e. p. 351. Of course, the directions must be reversed; it is in the *upward* not the downward direction that these strands appear to end blindly.

and shows very clearly the great dilatation of the rays in this region, and the corresponding thinning out of the tracheal bands, which here form a network, enclosing the great rays. The structure of the inner ends of the rays



TEXT-FIG. 5. Tangential section of secondary wood, showing the medullary rays. Where the rays are larger the plane of section approaches the pith.  $\times$  about 25. Slide 448 (G.T.G.).

appears to be identical with that of the pith. The rays in this part have run together vertically and are often of great height. In other words, when the formation of secondary wood began, the parenchymatous elements were predominant.

The radial section shows little or nothing of the pitting on the secondary tracheides—the preservation, as often happens in this Kentucky material, is too imperfect for such details to be recognizable. The medullary rays show the usual muriform structure in radial section.

#### DIAGNOSIS.

The characters of *Archaeopitys Eastmanii* were summed up in the Kentucky paper (p. 352). Clearly this summary requires amendment, in the light of the later observations, detailed above. We may now draw up the generic diagnosis as follows:

*Archaeopitys*, Scott and Jeffrey.

1. Pith (so far as shown) continuous, not discoid, traversed in all parts by scattered mesarch strands of primary xylem.

2. Numerous circum-medullary strands, also mesarch, present at the inner margin of the wood.

3. Certain of the circum-medullary strands (possibly all) passing out through the wood as leaf-traces, each leaf-trace acquiring its own zone of secondary wood, as it advances outwards.

4. Each leaf-trace giving off on its adaxial side, a reparatory bundle, which becomes a medullary strand.

5. Other medullary strands arising from the division of circum-medullary strands which do not immediately pass out as leaf-traces.

6. Medullary strands occasionally themselves dividing, as followed upwards.

7. Secondary wood of a dense, Cordaitan character, consisting of narrow, pitted tracheides, and of medullary rays, both multiseriate and uniseriate, usually of no great height.

8. Rays much dilated where they abut on the pith.

*Archaeopitys Eastmanii*, Scott and Jeffrey. The only known species. Characters those of the genus.

Boyle County, Kentucky, U.S.A.

Base of the Lower Carboniferous.

#### AFFINITIES.

This question was discussed in the Kentucky Memoir (p. 352). As regards the near relation to *Pitys* there can be no doubt. The plant clearly belongs to the family Pityeae. The difficulty is that Dr. Gordon's discovery of medullary strands in three species of *Pitys* tends to remove what once seemed the most evident distinction from our *Archaeopitys*. This point was referred to in my 'Studies in Fossil Botany' (5) pp. 260, 262. Dr. Gordon's full paper on *Pitys* still awaits publication, and until it appears any further discussion of the relation between the two genera seems superfluous. In the meantime, our original nomenclature is provisionally maintained, as *Archaeopitys Eastmanii* is the name under which the Kentucky plant is known.

Our knowledge of the ancient and remarkable allied genus *Callixylon* has been much advanced of late, chiefly by the researches of Dr. C. A. Arnold (1, 2, and 3). The structure of the wood turns out to have been ever more elaborate than was known before. Besides the well-known grouping of the main pitting in radial bands, the presence of pitted tracheides in the medullary rays has been demonstrated by Dr. Arnold in various species. This is a structural feature hitherto regarded as characteristic among Gymnosperms of certain of the higher Coniferae. The occurrence of ray-tracheides in a genus of an age at least as remote as the Upper Devonian

is certainly a surprising fact, showing how high a development the Gymnospermous type had attained in early times.

Dr. Arnold was also able to examine the structure of the phloem and bark in a species of *Callixylon*. He finds the phloem more simply constructed than in the Carboniferous genus *Mesoxylon* (2).

But for the purpose of comparison with *Archaeopitys* it is the question of medullary xylem which chiefly interest us. In *Callixylon* all the primary mesarch xylem-strands may be called circum-medullary. The process of emission of a leaf-trace, by division of a xylem-strand into the trace and a reparatory bundle, seems to be practically the same as in *Archaeopitys*. In *Callixylon Zaleskyi* the outgoing trace becomes surrounded by its own zone of secondary wood, just as in our plant ((1) pp. 29-31).

In *C. Newberryi*, many of the primary xylem-strands are separated from the secondary wood, and are completely surrounded by pith tissue (3) p. 214, Pl. IV, Fig. 2. This however, is scarcely a case of true medullary strands, for the separation from the wood is no greater than was originally observed in *Pitys antique* (4) p. 347, Pl. V, Fig. 16, before Dr. Gordon's discovery of deeply immersed strands.

Of greater interest, perhaps, is the occurrence of tracheides in the pith of species of *Callixylon*. 'They are for the most part, isolated and scattered, but frequently occur in groups of as many as 4 or 5, (1) p. 31 (Pl. VIII, Fig. 1): 1931, p. 214 (Pl. IV, Fig. 3). Their markings are spiral or scalariform. Dr. Arnold remarks that while medullary xylem-strands are not present 'their counterparts might be recognized in the isolated pith tracheides previously mentioned' (3) p. 224. This is true, but we cannot tell whether the medullary tracheides of *Callixylon* represent nascent or vestigial medullary strands or are merely a parallel development. When Dr. Gordon publishes in full his observations on the medullary strands (apparently somewhat inconstant) of *Pitys* we may be in a better position to discuss the question.

Dr. Arnold's investigation serves, as he says, to link *Callixylon* more closely with the Pityeae. The same is the position as regards *Archaeopitys*. It is a pity that the preservation leaves us rather ill-informed as to the details of the secondary wood. It has, however, a more Cordaitean character than that of *Callixylon* or *Pitys*.

#### SUMMARY.

The characters of *Archaeopitys* have already been summed up (p. 371) and need not be repeated. The genus is evidently closely allied to (possibly not even distinct from) *Pitys*; the strands immersed in the pith are perhaps more constant, and the wood is denser. The three genera, *Pitys*, *Callixylon*, and *Archaeopitys* form a definite, united group, distinct

from other Palaeozoic Gymnosperms. If we still include them within the order Cordaitales, this is only a provisional arrangement, for until we know something of their fructification, it is impossible to fix their true affinities.

The Pityeae are of special interest from their great antiquity, and it is remarkable that their most ancient genus, *Callixylon*, appears to have been, in the structure of the wood, the most highly organized of the family.

The five Text-figures were all drawn by that skilled draughtsman the late Mr. G. T. Gwilliam, F.R.A.S.

The photographs in Plate XV are the work of Mr. W. Tams of Cambridge.

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6. ———, and JEFFREY, E. C.: On Fossil Plants, showing Structure, from the Base of the Waverley Shale of Kentucky. Phil. Trans. Royal Soc. of London, Series B, ccv. 1914.

#### EXPLANATION OF PLATE XV.

Illustrating Dr. D. H. Scott's paper on *Archaeopitys Eastmanii*.

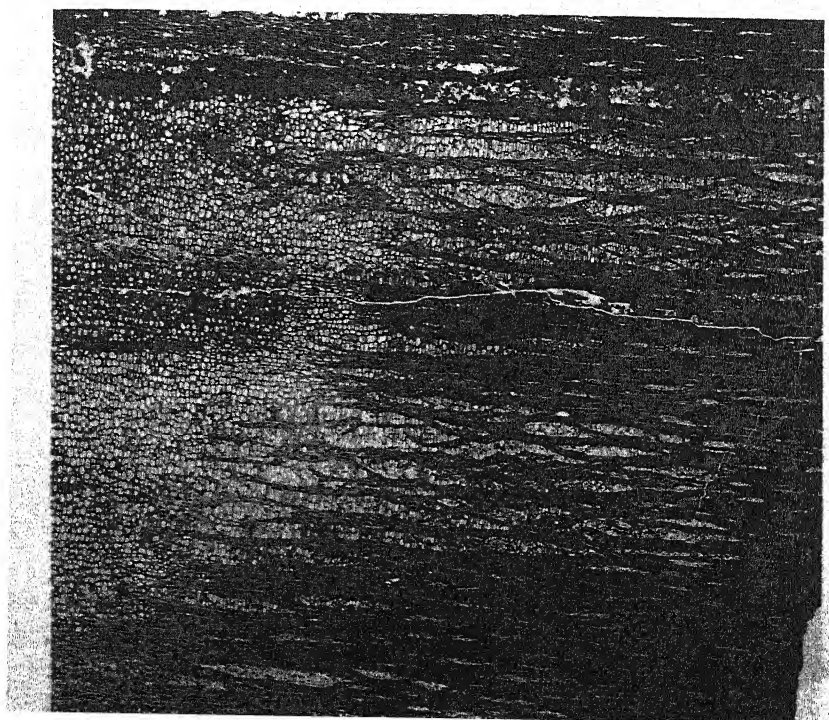
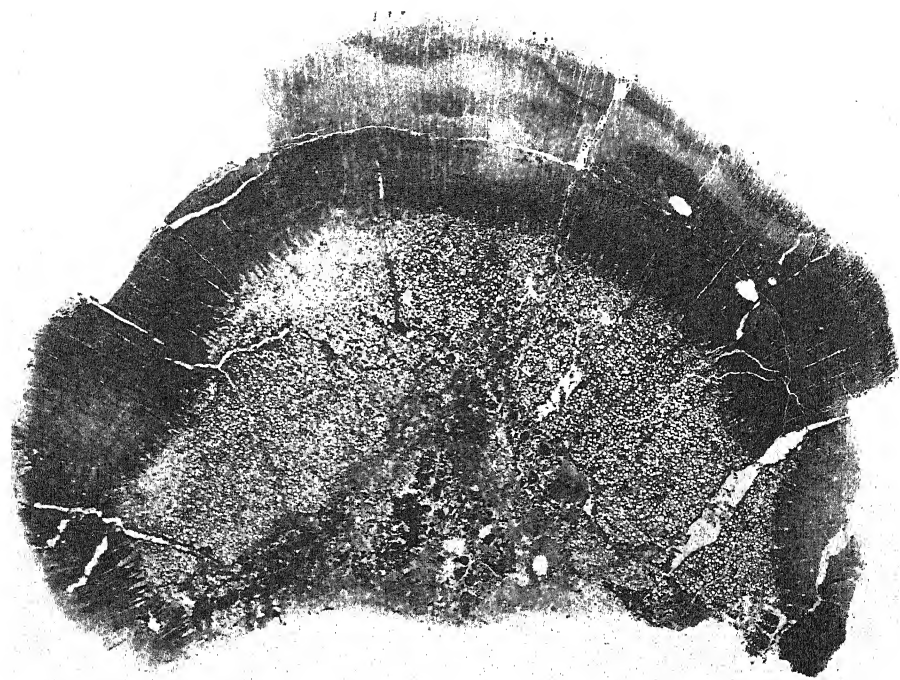
Fig. 1. General view of a transverse section showing the zone of secondary wood, surrounding the large pith, which is well preserved in the outer part, but disorganized towards the middle.  $\times$  about 3. Slide 427 (W.T.).

Fig. 2. Portion of another transverse section, enlarged, showing a well-preserved region of the pith with the adjacent secondary wood. Several circum-medullary strands are evident, and some of the small medullary strands can be recognized.  $\times$  about 10. Slide 429 (W.T.).

Fig. 3. Tangential section of wood and adjacent portion of pith. Note the great dilatation of the medullary rays as they approach the pith. The dark median band represents a circum-medullary strand.  $\times$  about 10. Slide 448 (W.T.).











# The Asci of *Lachnea scutellata*.

BY

H. C. I. GWYNNE-VAUGHAN

AND

H. S. WILLIAMSON.

With Plates XVI and XVII and eight Figures in the Text.

## INTRODUCTION.

IN November, 1931, ascocarps of *Lachnea scutellata*, an orange-red, discomycetous fungus growing on wood, were sent to us by Mr. F. W. Jane, B.Sc. Cultures were made, new fructifications developed, and ascospores were obtained and sown. The mycelium grew well on agar made up with the dung of various birds or mammals, suggesting that the substratum on which it flourishes in the wild state has been contaminated with excreta. Horse-dung agar with sodium carbonate proved satisfactory, and, as this medium is in general use in the laboratory, it was employed in the latter part of the work. When a plate became covered with hyphae it was transferred from the incubator at 25° C. to a sunny window. Fruiting requires moderate illumination, and the desired condition was obtained by allowing rays of direct sunlight to pass through a veil of green gauze.

Ascocarps developed in single spore culture, showing the fungus to be monoecious and homothallic.

Numerous multinucleate chlamydospores (Pl. XVI, Fig. 1) appeared in culture, and some of them soon became associated with knots of hyphae in which one or more larger filaments with denser contents could soon be distinguished. These filaments, in some cases at least, originate from a germinating chlamydospore (Pl. XVI, Fig. 2). They resemble the sexual branches observed in young apothecia, but, whereas the knot of hyphae may contain two or more such branches, only one is found at a later stage in the apothecium. It consists of about nine cells, the oogonium being usually the second or third from the end, perhaps because some distal cells have already disappeared.

The ascogenous hyphae, when they leave the oogonium, are very wide (Pl. XVI, Fig. 4), they branch freely, and here and there show expansions

of considerable extent, but ultimately give rise to the ordinary type of narrow filament on which asci are produced in the usual way.

#### THE DIVISION OF THE NUCLEI.

Though the nuclei of the ascus are not large, the details of their division are very clear, and they showed points of interest which justified further investigation. Both wild material and specimens grown in the laboratory were examined, but no difference was found in the nuclear structure. Our cordial thanks are due to Miss I. M. Wilson, M.Sc., for a most useful supply of ascocarps fixed in the field.

Towards the end of the meiotic prophase twelve gemini can be counted, two of them being larger than the remaining ten. In favourable cases the gemini are seen to be arranged in two groups (Pl. XVI, Fig. 7), each group including one long and five smaller units. In the earlier prophase, also, long and short chromosomes can be identified (Pl. XVI, Fig. 6), but the contents of the nuclei are too crowded to allow of accurate counts. At this stage the intertwined allelomorphs are recognizable, and a longitudinal fission in each is visible here and there. Differences of chromosome size have been noted in *Lachnea stercorea* (6).

In the metaphase (Pl. XVI, Fig. 8) the characteristic form is well shown by the two larger gemini, while in the anaphase (Pl. XVI, Fig. 9) ten small and two large chromosomes travel to each pole.

The spindle is intranuclear, the centrosomes being in contact with the nuclear membrane. They lie at first near together, but move apart as division proceeds (Pl. XVI, Figs. 7, 8) till they are separated by the diameter of the nucleus. Where, as in Pl. XVI, Figs. 9, 11, the centrosome appears within the nuclear area, it is actually in contact with the membrane either above or below.

The prophase of the second division (Pl. XVI, Fig. 10), like the preceding anaphase, shows ten short and two long chromosomes. The same numbers are visible in metaphase when, as in Pl. XVI, Fig. 11, an oblique view allows the whole circumference of the spindle to be examined. In the second anaphase, however (Pl. XVI, Fig. 12), only six chromosomes, one long and five short, pass to either pole.

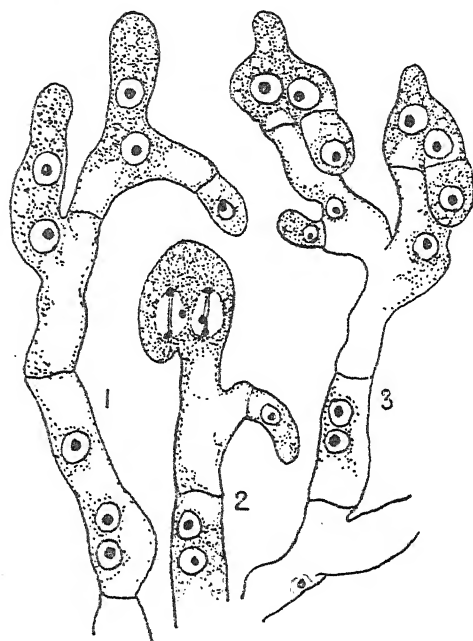
One long and five short chromosomes reappear in the third prophase, and already, at the stage represented in Pl. XVI, Fig. 13, show evidence of being longitudinally split. They pass on to the spindle (Pl. XVI, Fig. 14), the halves separate (Pl. XVII, Fig. 15), and are distributed to the poles. As in the previous telophase, one long chromosome with five others (Pl. XVII, Fig. 16) can be recognized at each pole.

The nuclear content is thus reduced from the twelve gemini, or twenty-four chromosomes, in the tetraploid definitive nucleus of the ascus, to six chromosomes in each of the haploid nuclei of the spores.

We have not examined mitosis in haploid nuclei other than those of the ascus, but we have seen diploid divisions in the oogonium and ascogenous hyphae. Pl. XVI, Fig. 5, shows prophase comparable to those represented in Pl. XVI, Figs. 10 and 13. There are twelve chromosomes, the diploid number, two being larger than the rest. The upper example is taken from an ascogenous hypha, the lower from the oogonium seen in general view in Pl. XVI, Fig. 4. Here all nuclei in the ascogenous hyphae are in process of division but, in the oogonium, a group of inactive nuclei may be noted. A similar condition was observed in *Pyronema confluens* (8) and in *Ascobolus magnificus* (9), both forms with normal fertilization, whereas, during mitosis in the oogonium of the apogamous *Humaria granulata* (7), no resting nuclei could be seen. We have been inclined to regard the inactive nuclei as haploid individuals for which a nucleus of opposite sex has not been available. If this interpretation is correct, the presence of nuclei in interphase, while others are dividing in the oogonium of *L. scutellata*, suggests the occurrence in this fungus of normal syngamy. We have, however, seen no satisfactory evidence of the existence of antheridia, though, in view of the presence of more than one sexual branch in the early stages of development, we are not prepared to assert that they do not occur.

The division of the nuclei in *L. scutellata* was described by Brown (2) in 1911. He observed in the apothecium an archicarp of about nine cells with large, richly branched ascogenous hyphae, and saw mitosis in the latter and in the oogonium from which they arose, as well as in the asci. He recorded at all stages the occurrence of five chromosomes, about equal in size, but he mentions also (p. 290) the presence of granules 'as large or larger than the chromosomes', and bearing 'such a striking likeness to them that there may appear to be as many as six or seven chromosomes'. From this statement and an examination of his Fig. 36 we suspect that Brown observed five small chromosomes and one large one, such as we have seen in haploid nuclei, though, owing to the difference in size, he did not recognize the larger structure as a chromosome. Whereas, however, we have counted ten small chromosomes and two large in the divisions in the ascogenous hyphae and in the first telophase and second prophase in the ascus, and also two large and ten small gemini in the meiotic prophase, Brown saw five (or six if his granules be included) at all these stages. We should be inclined to suppose that he had examined a eupogamous form, in which the first, or sexual fusion and the brachymeiotic reduction had entirely disappeared, but for his account of the anaphase of the first division in the ascus. In respect of this stage he states on p. 291, and shows in Fig. 40, that 'as the chromosomes approach the poles all of them may again divide'. From this it appears possible that he may have been dealing with nuclei which, in the meiotic anaphase, were still diploid, as we

have found them to be. In these small objects it is only too easy to count fewer chromosomes than are actually present. As stated above, the spindle is intranuclear, we have found nothing corresponding to Brown's account of the late telophase in which the groups of chromosomes (p. 292) 'break through the nuclear membrane'.



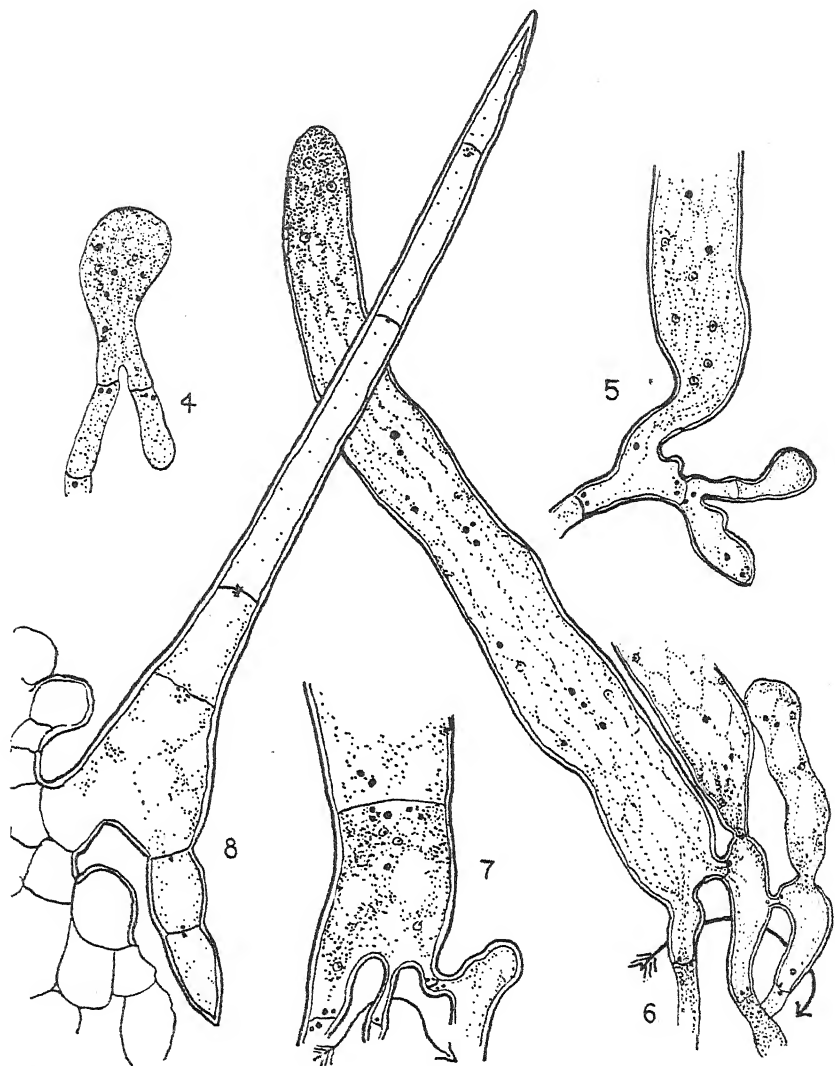
TEXT-FIGS. 1-3. Ascogenous hyphae of *Pyronema confluens*, showing intercalary cells from which the asci arise.  $\times 1,900$ .

#### THE DEVELOPMENT OF THE SPORES.

The spores of *L. scutellata* are characterized by small, regularly arranged excrescences of the outer coat. Binucleate and trinucleate spores are not uncommon, the number of spores in the ascus being correspondingly diminished. The ascus shown in Pl. XVII, Fig. 17, for example, has one large, trinucleate spore and five others.

When the spore is first laid down its contents are almost homogeneous (Pl. XVII, Fig. 17), the cytoplasm is densely granular, the vacuoles are minute. The increase in the amount of cytoplasm does not, however, keep pace with the enlargement of the spore, and soon (Pl. XVII, Fig. 18) numerous vacuoles can be seen. Outside, the epiplasm is becoming concentrated about the periphery of the spores. At the stage reached in Pl. XVII, Fig. 19, the epiplasm is less abundant, the outer walls of the spores show markings, the vacuoles within the spores are larger and more symmetrically arranged round the margin of the cytoplasm. The two middle





TEXT-FIG. 4. Young hair of *L. scutellata* developing from intercalary cell of sheath hypha.  $\times 700$ .

TEXT-FIG. 5. Part of older hair of *L. scutellata* from sub-terminal cell. The tip of the filament has branched.  $\times 700$ .

TEXT-FIG. 6. Three hairs of *L. scutellata* arising from adjacent cells. Beyond the cell producing the youngest hair is the terminal cell of the parent filament. The direction of growth of the latter is indicated by an arrow. The cell producing the middle hair shows a downwardly directed branch.  $\times 700$ .

TEXT-FIG. 7. Part of trifurcate hair of *L. scutellata*. A branch has been thrown out from the middle of the basal cell. The direction of growth of the parent hypha is indicated by an arrow.  $\times 700$ .

TEXT-FIG. 8. Hair from the sheath of the ascocarp of *L. stercorea*. The type of development is similar to that shown in Text-fig. 4.  $\times 700$ .

spores of this figure are in median section, the outer ones in tangential view. Two spores from an ascus at a somewhat later stage of development are represented in Pl. XVII, Fig. 20, in surface view. Seen thus the vacuoles form a continuous pattern, each beginning to assume a hexagonal outline by mutual pressure. In the nearly mature spore (Pl. XVII, Fig. 21) the hexagonal pattern is more marked, and the triangular masses of cytoplasm where three vacuoles meet are seen to correspond with the protrusions of the episporic. The same relation between the episporic markings and the cytoplasm delimiting the vacuoles can be seen also, though less clearly, in the middle spores of Pl. XVII, Fig. 19. It would appear that the external protuberances on the spore coat are derived from the epiplasm, and that they bear a definite relation to the strands of cytoplasm running between the nucleus and the periphery of the spore. We have examined corresponding stages in *Lachnea stercorea*, a form with smooth spores. In this species there are two large vacuoles in the long axis of the spore, one on either side of the nucleus. No peripheral vacuoles are present.

#### THE HAIRS OF THE SHEATH.

The asci and paraphyses of *L. scutellata* are surrounded, though never completely enclosed, by a somewhat solid sheath beset with large, pointed, septate hairs, many of which show a bifurcate base. Bifurcate hairs and cystidia appear to be of common occurrence. They were described by Cooke (8) for *Peniophora* in 1879, by Smith (11) for *Gomphidius* in 1881, by Boudier (1) for *Ciliaria* in 1896, and by Masee (10) in 1897 for species of *Lachnea* as well as for several other genera. Masee's account is of historical interest. When it was written Dangeard (4) had lately observed the fusion in the ascus, having regarded it at first as the union of nuclei from two independent cells. Masee is concerned to show that the fusion in the ascus is not sexual by demonstrating a similar process in the origin of bifurcate hairs. Having in mind these earlier records, we had no difficulty in finding examples (Text-figs. 4-7) of forked hairs, and we agree with Masee in regarding the method of origin as closely similar to that of the ascus, though in neither case is there a union of separate cells. Dangeard (4), in 1894, had already shown, as Masee was aware, that the ascus may be formed by the bending over of the tip of an ascogenous hypha and the upward growth of the subterminal cell. This has been almost universally confirmed, and has recently been shown (8, 9) to be a common method of development in the earlier growth of the ascogenous hyphae. In *Pyronema confluens*, for example, the septation of the ascogenous hypha (Text-fig. 1) gives rise to a uninucleate terminal cell, a uninucleate or trinucleate basal cell and one or more intervening cells with two nuclei each. Any of the binucleate cells may grow out (Text-figs. 1, 2)

to form a new branch, and, if its growth is strong, the distal and proximal parts of the parent hypha may assume an almost parallel position. The branch may grow onwards, or may bend over (Text-fig. 3), and give rise to an ascus.

The same type of growth appears to be responsible for the bifurcate hairs of *L. scutellata*. They arise from the middle cells of a filament of the sheath, which are apt to be richest in food material, and their energetic growth may distort the hypha from which they arise, so that, even when the hair is young (Text-fig. 4), the stalk and tip may lie almost parallel. Like other cells of the sheath, the young hair is multinucleate, differing in this respect from the young ascus, which contains only two nuclei. Additional branches may grow out (Text-figs. 5-7), either as lateral supports or in search of further nutriment, and thus the trifurcate appearance noted by Massee (Text-fig. 7), or even a larger number of forks may sometimes be seen. The relatively free branching may doubtless be associated with the multinucleate character of the cells.

An examination of old slides of *Lachnea stercorea* (5) reveals that the method of development of the hair (Text-fig. 8) is the same in that species as in *L. scutellata*.

#### SUMMARY.

1. The mycelium of *L. scutellata* grows freely, giving rise to chlamydospores, and producing ascocarps in single spore culture.

2. The oogonium is usually the third cell from the tip of a sexual branch of about nine cells. It gives rise to large ascogenous hyphae. Their nuclei divide simultaneously, showing twelve chromosomes, the diploid number.

3. In the definitive nucleus of the ascus twelve gemini are present, twelve chromosomes pass to each pole in the telophase, and twelve appear in the prophase of the second division. On the spindle of the second division the chromosomes separate, six passing to each pole. The third division in the ascus shows at all stages six chromosomes, the haploid number. Brachymeiosis is here accomplished in the second division.

4. One of the six chromosomes of the haploid group is noticeably longer than the others. In the meiotic prophase two long gemini are seen, and two long chromosomes in the divisions of the diploid nuclei.

5. The cytoplasm of the young spores is densely granular, but, as the spore enlarges, vacuoles appear. They become arranged round the periphery, and finally, by mutual pressure, assume a hexagonal form as seen from without. The strands of cytoplasm running between the vacuoles from the nucleus to the periphery seem to be concerned with the deposit of thickenings derived from the epiplasm on the outer face of the spore.

Peripheral vacuoles are not found in *L. stercorea*, a smooth spored species.

6. The bifurcate hairs arise from the intercalary cells of sheath hyphae, which become recurved, giving the hair its characteristic forked appearance.

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#### EXPLANATION OF PLATES XVI AND XVII.

Illustrating Professor Dame Helen Gwynne-Vaughan and Mrs. H. S. Williamson's paper on 'The Asci of *Lachnea scutellata*'.

##### PLATE XVI.

Fig. 1. Hypha bearing chlamydospores.  $\times 700$ .

Fig. 2. Young sexual branch of three cells.  $\times 700$ .

Fig. 3. Older branch of seven cells.  $\times 700$ .

Fig. 4. Part of a sexual branch enclosed in the ascocarp. The oogonium is giving rise to ascogenous hyphae. The nuclei in the latter, as well as most of those in the oogonium, are in the late prophase of mitosis.  $\times 700$ .

Fig. 5. Two nuclei in late prophase. The lower is from the section shown in Fig. 4 of the oogonium, the upper from a neighbouring ascogenous hypha.  $\times 2,600$ .

Fig. 6. Long and short chromosomes from the definitive nucleus of the ascus. At the ends of the two chromosomes associated to form the longest of these three gemini traces of the longitudinal fission can be seen.  $\times 2,600$ .

Fig. 7. Late prophase of the first meiotic division. The twelve gemini lie in two groups, with one short individual and five long in each. The spindle can be recognized at the lower end of the nucleus.  $\times 2,600$ .

Fig. 8. Metaphase of the first meiotic division. Two large gemini of characteristic form and ten others can be seen on the spindle. The spindle has not yet reached its full extent and is still lateral.  $\times 2,600$ .

Fig. 9. First meiotic anaphase; two long and ten short chromosomes on the way to each pole.  $\times 2,600$ .

Fig. 10. Prophase of second meiotic division. Ten short and two long chromosomes can be seen in the lower nucleus; in the upper, nine of the short chromosomes are visible, one of the long chromosomes is clear while the other is partly obscured by the nucleolus.  $\times 2,600$ .

Fig. 11. Second metaphase. In the upper nucleus the chromosomes cannot be counted, in the lower the spindle is cut across, so that one looks obliquely into one half of it, ten small chromosomes and two large ones showing in the position of the tips of the spokes of a half open umbrella.  $\times 2,600$ .

Fig. 12. Second anaphase. One long and five short chromosomes are visible at each pole. The second reduction is complete.  $\times 2,600$ .

Fig. 13. Prophase of the third division in the ascus. In the uppermost nucleus the chromosomes are crowded together, in each of the three others one long chromosome and five short can be seen. The daughter chromosomes are already recognizable.  $\times 2,600$ .

Fig. 14. Late metaphase of third division. In the uppermost nucleus five small chromosomes and one large are visible, in the second nucleus one, and in the third two, of the small chromosomes have divided, while in the fourth nucleus, in which only one side of the spindle is represented, the daughter chromosomes of the large chromosome and of three of the small ones can be seen.  $\times 2,600$ .

#### PLATE XVII.

Fig. 15. Anaphase of third division in the ascus. In the third nucleus from the top one large chromosome is visible travelling to each pole while the ten smaller ones are scattered about the spindle.  $\times 2,600$ .

Fig. 16. Third telophase. The one long and five short chromosomes are particularly well seen in the nucleus nearest the bottom of the ascus.  $\times 2,600$ .

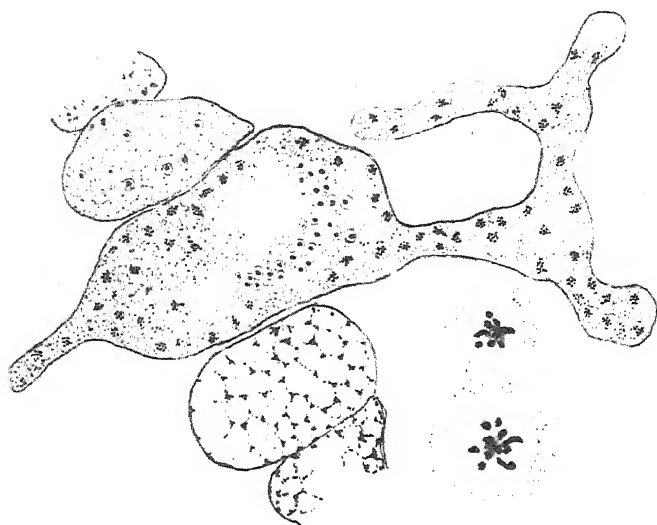
Fig. 17. Ascus with one trinucleate spore and five others. The spore plasma is dense, the epiplasm is collected round the spores.  $\times 1,600$ .

Fig. 18. Three spores from an older ascus, the spores have enlarged and vacuoles have appeared in the cytoplasm.  $\times 1,600$ .

Fig. 19. Four spores showing excrescences on epispor. In the two middle nuclei, in median section, the vacuoles are seen to be symmetrically arranged about the periphery.

Fig. 20. Two spores from a rather older ascus, showing the vacuoles in surface view.  $\times 1,600$ .

Fig. 21. A nearly mature spore, the vacuoles by mutual pressure have assumed a hexagonal form, their corners corresponding to the thickenings of the epispor.  $\times 1,600$ .

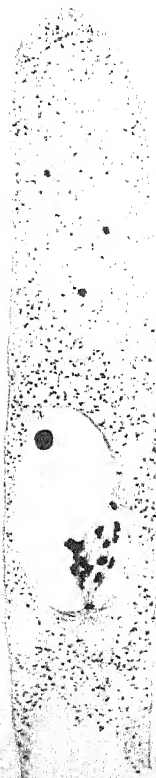
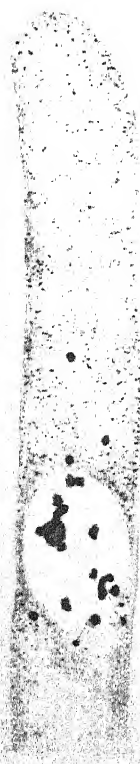


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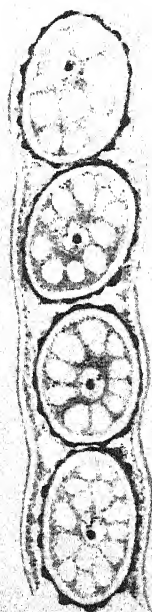








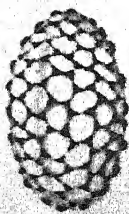
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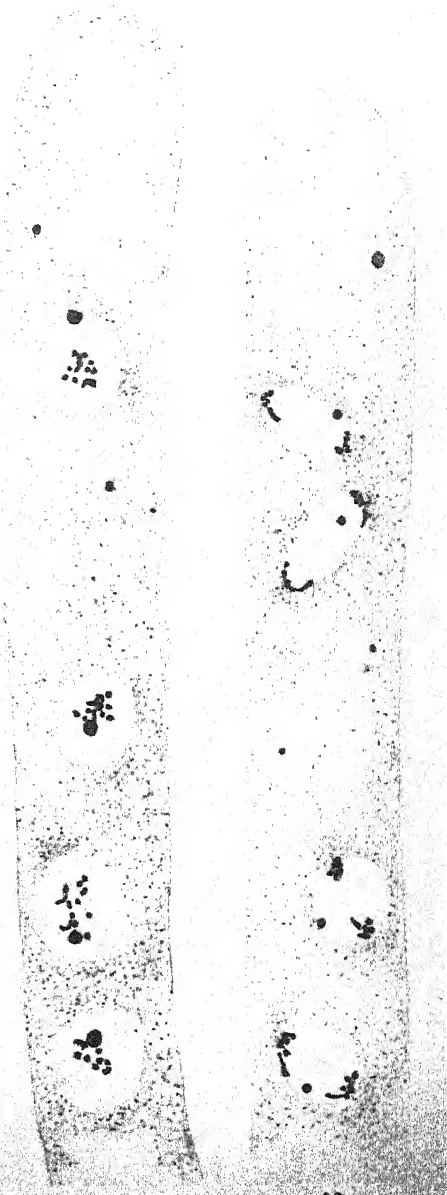
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# Studies in the Genera *Cytosporina*, *Phomopsis*, and *Diaporthe*.

## IV. On the Pathogenicity of certain Strains of *Phomopsis* and *Diaporthe*.

BY

S. N. DAS GUPTA, PH.D.

With five Figures in the Text.

### CONTENTS.

	PAGE
I. INTRODUCTION . . . . .	385
II. MATERIAL AND METHOD . . . . .	385
III. PATHOGENICITY OF <i>Phomopsis</i> and <i>Diaporthe</i> . . . . .	387
IV. COMPARISON OF RESULTS OBTAINED WITH <i>Phomopsis</i> , <i>Diaporthe</i> , and <i>Cytosporina</i> <i>ludibunda</i> . . . . .	397
V. DISCUSSION . . . . .	398
VI. SUMMARY . . . . .	400
LITERATURE CITED . . . . .	400

### I. INTRODUCTION.

THE previous paper (2) dealt exclusively with the relative powers of *Cytosporina ludibunda* and its saltants to attack the apple fruit. In view of the close resemblance observed in culture between these saltants and certain authentic species of *Phomopsis* and the suggested affinity between *C. ludibunda* and *Diaporthe perniciosa* it was thought advisable to extend the work to various available species and strains of *Phomopsis* and *Diaporthe*. The present paper embodies the results obtained in this further work and provides a comparison of the data accumulated during the entire investigation on attacking power.

### II. MATERIAL AND METHOD.

Bramley's Seedling apples were used in this investigation in 1928, and Worcester Pearmain apples in 1929. In Bramley's the 'cyclic' method of

inoculating apples with series of strains, described in the previous paper (2) has been employed.

The estimation of 'attacking power' of strains is based on the rate of radial advance calculated from the amount of rot produced by the inoculated strains (4). Wherever possible the data have been statistically analysed (3). As before, the differences between strains are regarded as significant only when the odds against the result being fortuitous exceed 100:1.

A complete list of the strains used in the investigation together with the name of their natural host, and the sources from which they were obtained is given below. For their morphological character see ((1) pages 360, 369-71, 375).

### 1. *Cytosporina*.

Strain.	Host.	Obtained from.
C <i>C. ludibunda</i> (Original strain)	Lane's Prince Albert Apples	A. S. Horne
CC A saltant from C		S. N. Das Gupta
CC <sub>2</sub> " " CC		" "
CA <sub>1</sub> " " C	via CA	" "
CA <sub>2</sub> " " CA <sub>1</sub>		" "
CA <sub>3</sub> " " CA <sub>1</sub>		" "
CA <sub>4</sub> " " CA <sub>3</sub>		" "
MK " " C		" "

### 2. *Phomopsis*.

<i>P. californica</i> Fawcett	Lemon fruit and bark of lemon trees (California)	Baarn (Holland)
<i>P. citri</i> Fawcett	Citrus fruit (Florida)	" "
<i>P. conglanensis</i> Trav.	Branch of <i>Aesculus hippocastanum</i>	" "
<i>P. vexans</i> (Sacc. et Syd.) Harter	Fruit of <i>Solanum melonganum</i>	" "
<i>P. pseudotsugae</i> Wilson	Douglas Fir Tree	Wilson (Edinburgh)
<i>P. quercina</i> (Sacc.) Died	On branch of <i>Tennellia quercinus</i>	Baarn (Holland)
<i>P. mali</i>	Apple fruit	M. N. Kidd (Cambridge)
<i>C. ludibunda</i>	Apple fruit	" "

### 3 a. *Diaporthe* (Exclusive of *D. perniciosa*).

<i>D. arctii</i> (Lasch.) Nit.	Stem of <i>Arctium</i> sp.	Baarn (Holland)
<i>D. binoculata</i> (Ell.) Sacc.	Twig of <i>Ilex</i>	" "
<i>D. faginea</i> (Curr.) Sacc.	Twig of <i>Fagus grandifolium</i>	" "
<i>D. species</i>	<i>Carya glabra</i> (Liverwort)	" "

### 3 b. *Diaporthe perniciosa*.

<i>D. perniciosa</i>	Apple fruit	A. S. Horne
"	Prunus tree and apple fruit	Kew (Isolated by Cayley)
"	Apple fruit	M. N. Kidd (Cambridge)
"	Wood of a living plum tree	Marsh (Bristol)
"	Small canker of Victoria Plum	Nattrass (Bristol)

<sup>1</sup> Since *Cytosporina ludibunda* (Kidd), showed the character of *Phomopsis*, while cultured by the author in standard medium (1) it has been included in the *Phomopsis* series.

## III. PATHOGENICITY OF PHOMOPSIS AND DIAPORTHE.

## A. Bramley's Seedling Apples.

1. *Phomopsis*.

Each sample consisted of twenty apples which were inoculated by the 'cyclic' method, using seven species. The complete cycle was as follows:

Sample 1.	<i>P. californica</i> and <i>P. citri</i> .
Sample 2.	<i>P. citri</i> and <i>P. coneglanensis</i> .
Sample 3.	<i>P. coneglanensis</i> and <i>P. vexans</i> .
Sample 4.	<i>P. vexans</i> and <i>P. mali</i> .
Sample 5.	<i>P. mali</i> and <i>P. pseudotsugae</i> .
Sample 6.	<i>P. pseudotsugae</i> and <i>C. ludibunda</i> .
Sample 7.	<i>C. ludibunda</i> and <i>P. californica</i> .

Apples were inoculated 16th-19th October, and rotted tissue estimated 5th-21st November. The longest interval between estimation of the first and second samples inoculated with the same species was nine days (*P. citri*).

The data of mean radial advance obtained for each species on the basis of eighteen half-apples in a sample are given in Table I.

TABLE I.

*Mean Radial Advance, cm. per Day, of Strains of Phomopsis*  
(15° C.-18° C.)

## Bramley's Seedling Apples.

Fungus.	Average of mean radial advance in cm. per day of Samples I and II.
<i>P. coneglanensis</i>	0.1694
<i>P. mali</i>	0.0976
<i>P. californica</i>	0.0329
<i>C. ludibunda</i>	0.0324
<i>P. citri</i>	0.0191
<i>P. vexans</i>	0.0099
<i>P. pseudotsugae</i>	0.0034

It is seen that the strains differ greatly in their attacking power, ranging from *P. coneglanensis*, which is very active (average 0.1694 cm. per day) to *P. pseudotsugae*, in which the rate of attack is negligible (0.0034 cm. per day). The attacking power is seen to vary to a certain degree with sample.

For the purpose of analysis of variance there are 14 samples, each consisting of 18 half-apples, giving 251 degrees of freedom in all; made up of 6 for strains, 1 for grouping, 6 for interaction, and 238 for variance within samples (Error).

TABLE II.  
*Analysis of Variance. Phomopsis.*

	Degrees of freedom.	Sum of squares.	Variance.	S. D.	Log S. D.	<i>z</i> .
Strains . . . .	6	0.7856	0.1310	0.3619	-1.048	2.453
Grouping . . . .	1	0.0058	0.0058	0.0762	-2.574	0.927
Interaction . . . .	6	0.0241	0.0040	0.0533	-2.759	0.742
Variance within sample (Error) . . . .	238	0.2167	0.0009	0.0302	-3.501	

For  $n_1 = 6$  and  $n_2 = 238$  1 % point of value of  $z$  is about 0.5152.

For  $n_1 = 1$  and  $n_2 = 238$  1 % point of value of  $z$  is about 0.9462.

The high value of  $z$  obtained for strains indicates that taken as a whole the strains differ significantly in attacking power.

The difference between any pairs of strains can be ascertained from the differences of their mean values (Table I, column 2) given in Table III.

Since the S.D. for the whole experiment is 0.03, the S.E. of difference between any two pairs of strains is 0.0071, i.e.  $(\frac{0.03\sqrt{2}}{\sqrt{36}})$ . Any difference of means which exceeds 0.0213 is considered significant.

TABLE III.  
*Difference of Mean Values. Phomopsis.*

	<i>P. coneglanensis.</i>	<i>P. mali.</i>	<i>P. californica.</i>	<i>C. ludibunda.</i>	<i>P. citri.</i>	<i>P. vexans.</i>
<i>P. mali</i>	0.0718					
<i>P. californica</i>	0.1365	0.0647				
<i>C. ludibunda</i>	0.1370	0.0652	0.0045			
<i>P. citri</i>	0.1503	0.0785	0.0138	0.0133		
<i>P. vexans</i>	0.1595	0.0877	0.0230	0.0225	0.0092	
<i>P. pseudotsugae</i>	0.1660	0.0942	0.0295	0.0290	0.0157	0.0065

The degree of significance of the difference between various means is shown diagrammatically in Fig. 1.

It is evident from Fig. 1 that *P. coneglanensis* and *P. mali* differ significantly from all other strains in attacking power. The remaining strains do not differ among themselves, except in the following pairs, where difference is significant:

*P. californica* and *P. pseudotsugae*;  
*C. ludibunda* and *P. pseudotsugae*;  
*P. californica* and *P. vexans*;  
*C. ludibunda* and *P. vexans*.

The value of  $z$  for grouping (Table II) is below the 1 per cent. point, and is not significant.

A significant effect of sample is seen in the result of interaction, since the value of  $z$  (0.742) is higher than the 1 per cent. point (0.515). Detailed analysis shows that this significant value of interaction is mainly due to one

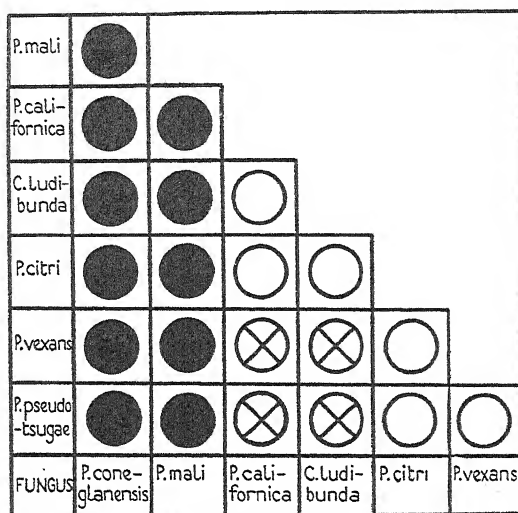


FIG. 1. Diagram showing degree of significance of difference between pairs of species of *Phomopsis* in Bramley's Seedling apples. S.E. = 0.0071.

- = a difference not exceeding 3SE (not significant)
- ⊗ = a difference between 3SE and 6SE (significant)
- = a difference between 6SE and 9SE (significant)
- = a difference exceeding 9SE (significant)

strain *P. californica*, where the difference between means of two samples (0.0281) is just three times the S.E. of difference (0.0098). No significance could be attached to differences shown by the remaining strains in the two samples.

## 2. *Diaporthe*.

Four different species of *Diaporthe* were used for inoculation, and the complete cycle was as follows :

- Sample 1. *D. arctii* and *D. binoculata*.  
 Sample 2. *D. binoculata* and *D. faginea*.  
 Sample 3. *D. faginea* and *D. species*.  
 Sample 4. *D. species* and *D. arctii*.

Apples were inoculated between Oct. 23–Oct. 25, and rotted tissue estimated between Nov. 5 and Nov. 21. The longest interval between estimates of the first and second sample inoculated with the same strain was twenty days (*D. binoculata*).

The data of mean radial advance obtained for each strain on the basis of eighteen half-apples are given in Table IV.

TABLE IV.

*Mean Radial Advance in cm. per Day of Species of Diaporthe*  
(15° C.–18° C.).

Bramley's Seedling Apples.

Fungus.	Average of mean radial advance in cm. per day of Samples I and II.
<i>D. faginea</i>	0.0243
<i>D. species</i>	0.0138
<i>E. arctii</i>	0.0065
<i>D. binoculata</i>	0.0052

It is seen that the strains differ in attacking power. *D. faginea* is the most active (average 0.0243 cm. per day), and *D. binoculata*, the least active (average 0.0052 cm. per day).

Analysis of variance shows that the value of  $s$  for strains (1.763) is three times that of the 1 per cent. point (0.5152), indicating that the strains differ significantly in attacking power.

The difference between the mean values of any pairs of strains (Table IV, column 2) can be ascertained from Table V. Since the S.D. for the experiment is 0.009, the S.E. of difference between any two pairs of strains is 0.002. The minimum value for significance is 0.006.

TABLE V.

*Difference of Mean Values.* Diaporthe.

Bramley's Seedling Apples.

Fungus.	<i>D. faginea.</i>	<i>D. species.</i>	<i>D. arctii.</i>
<i>D. species</i>	0.0105		
<i>D. arctii</i>	0.0178	0.0073	
<i>D. binoculata</i>	0.0191	0.0086	0.0013

The degree of significance of the difference between various means is shown diagrammatically in Fig. 2.

It is clear from Fig. 2 that all the pairs, except that of *D. arctii* and *D. binoculata*, differ significantly in attacking power.



The analysis further shows that the value of  $z$  for grouping is clearly non-significant, but the differential effect of samples on strain is brought







D.species			
D.arctii			
D.bino- culata			
SPECIES	D.faginea	D.species	D.arctii

FIG. 2. Diagram showing degree of significance of difference between pairs of species of *Diaporthe* in Bramley's Seedling apples. S.E. = 0.002. For key to signs see page 389.

out by the significant value for interaction (value of  $z$  is higher than 1 per cent. point).

### 3. *Diaporthe perniciosa*.

Five strains of *D. perniciosa* were used for inoculation, and the complete cycle was as follows :

- Sample 1. *D. perniciosa* (Horne) and *D. perniciosa* (Kidd).
- Sample 2. *D. perniciosa* (Kidd) and *D. perniciosa* (Cayley).
- Sample 3. *D. perniciosa* (Cayley) and *D. perniciosa* (Natrass).
- Sample 4. *D. perniciosa* (Natrass) and *D. perniciosa* (Marsh).
- Sample 5. *D. perniciosa* (Marsh) and *D. perniciosa* (Horne).

Apples were inoculated between Oct. 26 and Nov. 2, and rotted tissue estimated between Nov. 9 and Nov. 17.

The data of radial advance obtained for each strain on the basis of eighteen half-apples in a sample are given in Table VI.

TABLE VI.

Mean Radial Advance in cm. per Day of Strains of *D. perniciosa*.  
(15° C–18° C.).

Bramley's Seedling Apples.

Fungus.	Average of the mean radial advance in cm. per day of Samples I and II.
<i>D. perniciosa</i> (Cayley)	0.1600
" (Marsh)	0.1355
" (Natrass)	0.0855
" (Horne)	0.0734
" (Kidd)	0.0594

There is a remarkable difference in the attacking power of *D. pernicios*. *D. pernicios* (Cayley) being the most active and *D. pernicios* (Kidd) the least active, with the others as intermediate.

The analysis of variance shows that the value of  $\alpha$  (2.019) is three times the 1 per cent. point (0.706), indicating that the differences observed for various strains of *D. pernicios* are significant.

TABLE VII.

*Difference of Mean Values. D. pernicios.*

## Bramley's Seedling Apples.

Strain.	<i>D. pernicios</i> (Cayley).	<i>D. pernicios</i> (Marsh).	<i>D. pernicios</i> (Nattrass).	<i>D. pernicios</i> (Horne).
<i>D. pernicios</i> (Marsh)	0.0245			
" (Nattrass)	0.0745	0.0500		
" (Horne)	0.0866	0.0621	0.0121	
" (Kidd)	0.1006	0.0721	0.0261	0.0140

The degree of significance of the difference between various means is shown diagrammatically in Fig. 3. The S.E. of difference of means is 0.0081 and the minimum value of the difference between strains must be 0.0243 to be significant.

Fig. 3 shows that the strains *D. pernicios* (Cayley), and *D. pernicios* (Marsh) are significantly different from all the other strains. Among the remaining three strains tested, there is significant difference only between *D. pernicios* (Natt.) and *D. pernicios* (Kidd).

The value of  $\alpha$  for grouping was again non-significant, but the differential effect of samples on strain is brought out by the significance of the value of  $\alpha$  for interaction which is mainly due to one strain, *D. pernicios* (Natt.) for which the difference between means of two samples (0.0257) is just three times the S.E. of difference (0.0076).

## B. Worcester Pearmain Apples.

Apples were inoculated on both sides with the same strain. Since only a limited number of apples were available, each sample consisted of seven individuals.

All the three series used in Bramley's Seedling apples, viz., *Phomopsis*, *Diaporthe*, and *D. pernicios*, have been tested. The mean data of radial advance obtained for all the sets are given in Table VIII.

It will be seen from Table VIII that, as in Bramley's, the members of each series differ greatly in attacking power. The difference between extremes is very considerable in the *Phomopsis* and *D. pernicios* series, and least in the *Diaporthe* series.

The results of the analysis of variance for the data of these three series, are given separately in abridged form in Table IX, together with











D. pern. (Marsh)				
D. pern. (Nattr)				
D. pern. (Horne)				
D. pern. (Kidd)				
STRAIN	D. pern. (Cayley)	D. pern. (Marsh)	D. pern. (Nattr)	D. pern. (Horne)

FIG. 3. Diagram showing degree of significance of difference between pairs of strains of *Diaporthe perniciosa* in Bramley's Seedling apples. S.E. = 0.0081. For key to signs see page 389.

the S.E. of difference of means for each series in the last column of the Table.

TABLE VIII.

*Mean Radial Advance in cm. per Day of Species of Phomopsis and Diaporthe and Strains of D. perniciosa (15° C.-18° C.).*

Worcester Pearmain Apples.

	Fungus.	Mean radial advance in cm. per day.
<i>Phomopsis</i>	<i>P. conglanensis</i>	0.1143
	<i>P. mali</i>	0.0301
	<i>P. californica</i>	0.0222
	<i>C. ludibunda</i>	0.0124
	<i>P. citri</i>	0.0061
<i>Diaporthe</i>	<i>D. species</i>	0.0186
	<i>D. faginea</i>	0.0127
	<i>D. arctii</i>	0.0042
	<i>D. binoculata</i>	0.0031
<i>D. perniciosa</i>	<i>D. perniciosa</i> (Marsh)	0.0701
	" (Nattrass)	0.0598
	" (Horne)	0.0474
	" (Cayley)	0.0264
	" (Kidd)	0.0069

The high value of  $z$  obtained for *Phomopsis* and *D. perniciosa* (Table IX) indicates that the observed differences between strains are significant. The differences among the members of *Diaporthe* series is, however, not significant, since the value of  $z$  (0.6139) is below the 1 per cent. point (0.7757).

In order to find out the strains responsible for the significance of the

value of  $z$  in series *Phomopsis* and *D. perniciosa*, the data obtained for each were subjected to further analysis. For this, the difference of means was compared with the S.E. of difference (0.0137).

TABLE IX.

*Analysis of Variance. Phomopsis, Diaporthe, and D. perniciosa.*

		Degrees of freedom.	S.D.	$z$ .	1 % point.	S.E. (difference of means).
<i>Phomopsis</i>	Strain	4	0.114	+1.7709	0.6954	0.0102
	Error	30	0.019			
<i>Diaporthe</i>	Strain	3	0.019	+0.6139	0.7757	0.0056
	Error	24	0.011			
<i>D. perniciosa</i>	Strain	4	0.067	+0.9680	0.6954	0.0137
	Error	30	0.025			

Differences of means for strains of *D. perniciosa* are given in Table X, and those which exceed 0.0411 (three times S.E. of difference) are significant and will be found in block type.

TABLE X.

*Difference of Mean Values. Strains of D. perniciosa.*

Worcester Pearmain Apples.				
Strain.	<i>D. perniciosa</i> (Marsh).	<i>D. perniciosa</i> (Natrass).	<i>D. perniciosa</i> (Horne).	<i>D. perniciosa</i> (Cayley).
<i>D. perniciosa</i> (Natrass)	0.0124			
" (Horne)	0.0227	0.0103		
" (Cayley)	0.0433	0.0330	0.0206	
" (Kidd)	0.0623	0.0529	0.0405	0.0199

It will be seen that significant difference exists between the following pairs of strains:

1. *D. perniciosa* (Cayley), and *D. perniciosa* (Marsh).
2. " (Kidd), " (Marsh).
3. " (Kidd), " (Natrass).

In the *Phomopsis* series, it is found that the significance of the value of  $z$  is due to one strain alone, viz., *P. coneglanensis*, a strain significantly different from all the other strains. The differences observed between all the other strains are within the experimental error.

With the object of comparing the results obtained with Bramley's Seedlings (1928), and Worcester Pearmain (1929), a fresh mean was calculated for Bramley's Seedlings on the basis of seven apples in a sample instead of eighteen.

The mean values expressed in terms of radial advance in cm. per day, together with differences of means (in block type where they are significant) are given for the two varieties in Table XI.

TABLE XI.

*Bramley's Seedlings and Worcester Pearmain. Mean Values and Differences.*

Fungus.	Mean radial advance in cm. per day.		Differences.
	Bramley's.	Worcesters.	
<i>P. coneglanensis</i>	0.1364	0.1143	0.0221 $\pm$ 0.0192
<i>P. mali</i>	0.1037	0.0301	0.0736 $\pm$ 0.0134
<i>C. ludibunda</i>	0.0318	0.0124	0.0194 $\pm$ 0.0052
<i>P. californica</i>	0.0256	0.0222	0.0034 $\pm$ 0.0085
<i>P. citri</i>	0.0162	0.0061	0.0101 $\pm$ 0.0017
<i>D. faginea</i>	0.0238	0.0127	0.0111 $\pm$ 0.0052
<i>D. species</i>	0.0177	0.0186	0.0009 $\pm$ 0.0068
<i>D. arctii</i>	0.0067	0.0042	0.0025 $\pm$ 0.0023
<i>D. binoculata</i>	0.0050	0.0031	0.0019 $\pm$ 0.0006
<i>D. perniciosa</i> (Cayley)	0.1580	0.0268	0.1312 $\pm$ 0.0212
" (Marsh)	0.1276	0.0701	0.0575 $\pm$ 0.0130
" (Natrass)	0.0823	0.0598	0.0225 $\pm$ 0.0114
" (Horne)	0.0665	0.0474	0.0191 $\pm$ 0.0113
" (Kidd)	0.0614	0.0069	0.0545 $\pm$ 0.0063

The differences in radial advance seen for the two varieties (Table XI) is markedly significant, since it has been found by analysis of variance that the value of  $z$  for varieties is 1.037, which exceeds the 1 per cent. point (0.9784). This result is contrary to that obtained with the *Cytosporina* saltants (2) where the value of  $z$  for varieties proved insignificant. The differential effect of varieties for this experiment, however, is seen in interaction, where the value of  $z$  (2.468) greatly exceeds the 1 per cent. point (0.3908) and is therefore markedly significant. In this respect the results conform to that obtained for *Cytosporina* saltants.

It should be remembered that the data compared here for the varieties are taken from experiments carried out in different years, and the result for variety probably includes an effect of age of apples. It has been shown in the previous paper (2) that the rate of attack of fungi changes significantly with increasing age of fruit.

The degree of significance to be attached to the difference in the rate of invasion for an individual strain in the two varieties may be ascertained from Table XI where the S.E. of difference of means is given for each fungus. In *P. citri*, *P. mali*, *C. ludibunda*, *D. binoculata*, *D. perniciosa* (Cayley), *D. perniciosa* (Kidd), and *D. perniciosa* (Marsh), the differences

are significant; the degree of significance varying with strains. The differences are not significant for the following: *P. coneglanensis*, *P. californica*, *D. faginea*, *D. arctii*, *D. species*, *D. perniciosa* (Horne), and *D. perniciosa* (Natrass).

#### IV. COMPARISON OF RESULTS OBTAINED WITH *PHOMOPSIS*, *DIAPORTHE*, AND *CYTOSPORINA LUDIBUNDA*.

The data compared are derived from the same population of apples, and although the experiments with sets of strains were carried out independently, the experimental conditions were similar in all the sets.

##### *Bramley's Seedling Apples.*

Data of mean radial advance for strains of *C. ludibunda* are given in one column, and those for strains of *Phomopsis* and *Diaporthe* in another parallel column in Table XII, arranged in ascending order of attacking power. The strains with similar attacking power have been grouped together.

TABLE XII.

*Mean Radial Advance in cm. per Day. Phomopsis, Diaporthe, and Cytosporina ludibunda in Bramley's Seedling Apples.*

Group.	<i>Cytosporina ludibunda.</i>	<i>Phomopsis and Diaporthe.</i>	
		Mean radial advance in cm. per day.	
1.	CC <sub>2</sub>	0.0023	0.0034 <i>P. pseudotsugae</i> 0.0052 <i>D. binoculata</i> 0.0065 <i>P. arctii</i> 0.0099 <i>P. vexans</i>
2.	C	0.0171	0.0138 <i>D. species</i>
	CA <sub>4</sub>	0.0180	0.0191 <i>P. citri</i>
	CC	0.0191	0.0243 <i>D. faginea</i>
3.	MK	0.0331	0.0324 <i>C. ludibunda</i> (Kidd) 0.0329 <i>P. californica</i>
4.	CA <sub>3</sub>	0.0471	
	CA <sub>1</sub>	0.0573	0.0594 <i>D. perniciosa</i> (Kidd)
5.	CA <sub>2</sub>	0.0721	0.0734 <i>D. perniciosa</i> (Horne) 0.0855 " (Natrass)
6.			0.0976 <i>P. mali</i> 0.1355 <i>D. perniciosa</i> (Marsh) 0.1600 " (Cayley) 0.1694 <i>P. coneglanensis</i>

It is seen from Table XII that many of the species and strains of *Diaporthe* and *Phomopsis* tested fall within the range of attacking power shown by the saltants of *C. ludibunda*. Thus four species may be grouped with CC<sub>2</sub>; three species with C<sub>1</sub>; CA<sub>4</sub> and CC, and so on. The only

strains which appear to be distinctly more active than *Cytosporina* saltants are *P. coneglanensis*, *P. mali*, and the strains of *D. perniciosa* obtained from Marsh and Cayley.

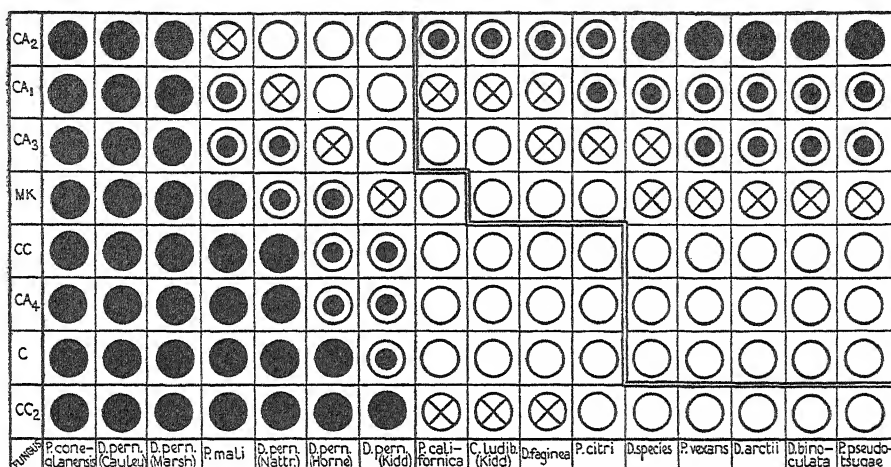


FIG. 4. Diagram showing degree of significance of difference between *Cytosporina ludibunda* (parent and saltants) and species of *Phomopsis* and *Diaporthe* in Bramley's Seedling apples. In pairs on the left of the double line, the mean radial advance of the *Cytosporina* strain is less than that of the other fungus; in pairs on the right of the double line, the mean radial advance of the *Cytosporina* strains exceeds that of the other fungus. S.E. = 0.0062. For key to signs see page 389.

The significance of the observed difference has been tested in the usual way. The S.D., given by analysis of variance for the entire set of strains and based on thirty-six half-apples, is 0.0265 and the S.E. of the difference of means, calculated from this value, is 0.0062. Differences greater than 0.0186 are regarded as significant.

The result of the analysis shows:

1. All the strains in group 6, are significantly more active than *Cytosporina* saltants; otherwise there is close correspondence between *Cytosporina* strains and strains of *Phomopsis* and *Diaporthe*.
2. The grouping in Table XII is artificial, and not based on significant difference between strains. Thus in group 5, *D. perniciosa* (Nattrass) differs from the saltants of all other groups, whereas *D. perniciosa* (Horne) of the same group, cannot be distinguished from CA<sub>1</sub> of group 4.

#### Worcester Pearmain Apples.

The data of mean radial advance calculated on the basis of seven apples in a sample, are given for *Cytosporina* in one column and those for *Phomopsis* and *Diaporthe* in another parallel column of Table XIII, arranged in ascending order of attacking power.

TABLE XIII.

*Worcester Pearmain Apples.*

*Mean Radial Advance in cm. per Day. Phomopsis, Diaporthe, and Cytosporina ludibunda.*

Group.	<i>Cytosporina ludibunda.</i>	<i>Phomopsis and Diaporthe.</i>
	Mean radial advance in cm. per day.	
1.		0.0031 <i>D. binoculata</i>
		0.0042 <i>P. arctii</i>
		0.0061 <i>P. citri</i>
		0.0069 <i>D. perniciosa</i> (Kidd)
		0.0124 <i>C. ludibunda</i> (Kidd)
	CC 0.0157	0.0127 <i>D. faginea</i>
	CC <sub>2</sub> 0.0167	0.0186 <i>D. species</i>
	C 0.0177	0.0222 <i>P. californica</i>
		0.0268 <i>D. perniciosa</i> (Cayley)
		0.0301 <i>P. mali</i>
		0.0474 <i>D. perniciosa</i> (Horne)
2.	MK 0.0542	0.0598 <i>D. perniciosa</i> (Nattrass)
3.	CA <sub>2</sub> 0.0759	0.0701 <i>D. perniciosa</i> (Marsh)
4.	CA <sub>4</sub> 0.0860	0.1143 <i>P. coneglanensis</i>

The significance of the observed difference between individual strains of *Cytosporina ludibunda* and those of *Phomopsis* and *Diaporthe* is shown in Fig. 5. The S.E. of difference for the experiment is 0.0118.

Analysis shows that in Worcester Pearmain, as in Bramley's, the attacking power of strains of *Phomopsis* and *Diaporthe* falls within the range of that shown by *Cytosporina* saltants. In contradistinction to the results in Bramley's, however, the saltant most active in Worcesters (CA<sub>4</sub>) is comparable to the most active of the *Phomopsis* series (*P. coneglanensis*).

## DISCUSSION.

The results obtained in this investigation show that so far as the power of attack on apples is concerned, *Cytosporina ludibunda* cannot be distinguished from the species of *Phomopsis* and *Diaporthe* studied. This similarity agrees with that observed in earlier cultural work in which the only critical character of any value was found to be the production of perithecia by certain strains of *Diaporthe perniciosa*.

The strains of *D. perniciosa* differ greatly in attacking power. In Worcester Pearmain apples the rate of invasion in cm. per day varies from 0.0069 (*D. perniciosa*, Kidd) to 0.0701 (*D. perniciosa*, Marsh). The corresponding values for *C. ludibunda* are 0.0517 (CC) and 0.0860 (CA<sub>4</sub>). The two series tend to run parallel in this respect. The differences between strains are more pronounced in Bramley's than in Worcester Pearmain.



None of the fungi tested differs significantly from the *Cytosporina* saltants in both Bramley's and Worcesters. In Bramley's four of them, *P. mali*, *D. pernicios* (Marsh), *D. pernicios* (Cayley), and *P. coneglanensis*

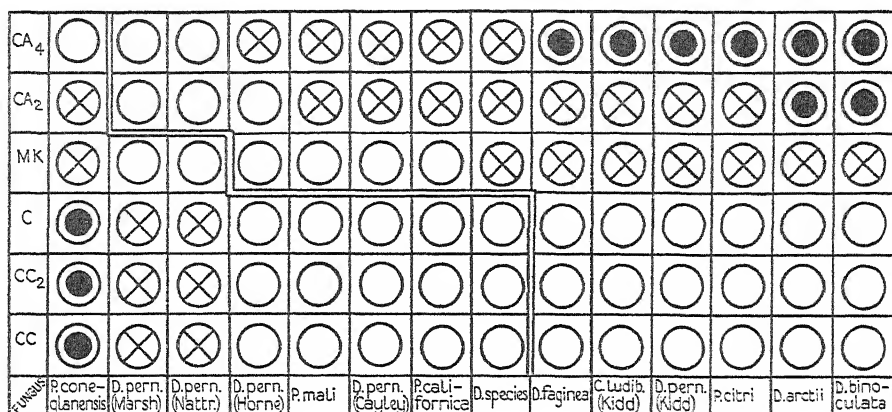


FIG. 5. Diagram showing degree of significance of difference between *Cytosporina ludiounda* (parent and saltants) and species of *Phomopsis* and *Diaporthe* in Worcester Pearmain apples. In pairs on the left of the double line, the mean radial advance of the *Cytosporina* strain is less than that of the other fungus; in pairs on the right of the double line, the mean radial advance of the *Cytosporina* strain exceeds that of the other fungus. S.E. = 0.0118. For key to signs see page 389.

are significantly different from *Cytosporina* and of these *P. coneglanensis* (0.1694), and *D. pernicios* (Cayley) (0.1600), are equally active. In Worcester Pearmain, however, the value for *P. coneglanensis* (0.1143) is higher than that for CA<sub>4</sub> (0.0860), the most active of the *Cytosporina* saltants, but the difference is not significant. All the species of *Diaporthe* and of *Phomopsis* therefore fall well within the range of variation of *Cytosporina*.

Attempts to arrange the strains in groups have proved somewhat unsatisfactory. Certain strains, such as *D. binoculata* and *D. arctii*, have consistently proved either medium or very weak; one strain, *P. coneglanensis*, has proved very active. The rest, while presenting a certain order with respect to a given variety of apple, are liable to vary in rate of attack from variety to variety; age of apple may also have a differential effect on attacking power of strains.

Owing to the various possible differential effects, it is difficult to make exact comparison of attacking power because observations made at any one time in the storage life of apples may prove misleading. Strains which are indistinguishable in early apples have been found to attack later ones at different rates. Again, strains which are very different on one variety may attack at nearly the same rate in others. As pointed out by Horne (5) 'The true comparison of strain must depend ultimately on the form of the curves representing in each case the change in time of the rate of invasion'.

## SUMMARY.

The investigation deals chiefly with the power of certain species of *Phomopsis* and of *Diaporthe* to attack the apple fruit and supplements that already described for *C. ludibunda* (2). The attacking powers of all the fungi investigated in this and the previous paper (2) are compared.

The strains of *D. perniciosa* differ widely, the range of variation agreeing approximately with that recorded for *C. ludibunda* and its saltants. The range of activity shown by the remaining species of *Diaporthe* (*D. binoculata*, *D. faginea*, &c.) is not extensive, and these may be classed with the least active strains of *C. ludibunda*.

The species of *Phomopsis* also show a wide range of variation falling within that found for saltants of *C. ludibunda*. Only the strain *P. coneglanensis* is significantly more active than *C. ludibunda* saltants (Bramley's), but it does not differ significantly from some strains of *D. perniciosa*.

Comparisons are rendered difficult by the effects of age of fruit and variety of apple on rate of attack by the fungi.

I wish to express my thanks to Professor V. H. Blackman and to Dr. A. S. Horne for their help and criticism.

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# The Mechanism of Water Conduction in the Musci considered in Relation to Habitat.

BY

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With twenty Figures in the Text.

## PART I. *Mosses growing in Wet Environments.*

A SURVEY of the publications of work on the Bryophyta shows that with very few exceptions investigation has been largely confined to the cataloguing of genera of different regions and habitats, and to the accurate identification of species. There are very few records of investigation on the physiology of the group, and apart from the work of Davy, (3) no information is available of any investigation on the connexion between the type of organization of a moss plant and its water supply. The work of Davy is confined very largely to an investigation of the modifications which occur in species when they are completely submerged in water or when they are grown in saturated atmospheres. From his observations Davy concludes that air saturated with water represents an optimum condition for growth and development for all mosses investigated. In saturated air development is more rapid, stems branch more freely, rhizoids are more numerous, leaves are smaller, cells are smaller and contain fewer but larger chloroplasts. It can be concluded therefore that a saturated atmosphere constitutes the ideal environment for mosses, but it is also obvious that the mosses of the world are not confined to such humid atmospheres, for there are some species which grow under dry conditions, and some even under conditions of extreme drought. The aim of the present work is to show how the organization of some of the most common mosses of the Gower Peninsula varies in relation to the water supply of their environments. Twenty-two species have been studied and these can be classified into three groups according to the degree of water present in their habitats.

A. Mosses growing in wet situations where there is an abundance of

both water and water vapour. (The present instalment of the work deals with mosses growing in such habitats.)

B. Mosses growing in damp situations where the supply of ground water is smaller, but where the atmosphere is still humid.

C. Mosses growing in dry situations where the substratum is dry and where the atmosphere is less humid.

It should be noticed, however, that even when the substratum is dry the low-growing habit of all forms studied ensures the retention of some water vapour around all parts of the plant.

A preliminary investigation was made of *Polytrichum commune*, and the result of work on this moss has already been published (2). Since the publication of this work and after the work reported in the present communication was completed, the author's attention was drawn to the work of Blaikley (1), contesting the author's conclusion that the main water supply of *P. commune* is derived by conduction over the external surface, and insisting that 'internal conduction of water sufficient to prevent wilting under the conditions of the experiment' occurs uniformly in this moss. The conditions of the experiment in Blaikley's work seem often, however, to involve such arrangements as the covering of the plants with a bell-jar, along with a dish of water—conditions not quite comparable with those of the natural habitat of the moss. The ascent of the internally conducted water is stated to take place in the central strand—though in what part of this tissue is not indicated. The present author has no doubt whatever of the movement of water in the hydrom of this strand, but finds that movement takes place downwards as well as upwards, and that the water, instead of entering at the base of the plant and passing steadily upwards, as Blaikley assumes, enters through the leaf-bases and finally through the apex of the shoot, and, diffusing from the leaf-bases laterally into the central strand, can there pass both up and down.

Most of Blaikley's experiments were performed with cut stems, whereas the author was concerned very largely with solving the question of absorption and conduction of water in whole plants in their natural condition, so that the problem of the initial entry of water into the stem was not met by Blaikley. Where the author used cut stems with the cut surface exposed to the liquid, some upward conduction internally was also demonstrated, as in Blaikley's experiments, though not so marked as Blaikley reports. It is, of course, not unlikely that where the liquid is applied directly to the cut surface of the hydrom of the central strand upward conduction will be more rapid than in the case of complete plants, where the water has to penetrate through an outer 'cortical' tissue, varying in extent and thickness of walls, before it can reach the central strand. In such whole plants if, as the author finds, the water can rise over the external surface and reach the relatively unthickened apical regions more

rapidly than it can penetrate through the cortex, it will enter at the apex, and the tendency will be for it to pass downwards through the central strand, and not upwards.

Blaikley's conclusion is that 'cut stems of *P. commune* absorb relatively large quantities of water', but it should be pointed out that the figures she gives for the amounts of water passing up internally represent only a fraction of the quantities which the author finds to pass up rapidly to the tip of the plant over the external surface. For instance, her maximum figures for three days are 0.4875 c.c., while the author finds that in a similar period 4.15 c.c. pass up over the external surface. Moreover, many of Blaikley's conclusions are based on results of potometer experiments, in which she estimates the loss of water from a leafy shoot by weight, and assumes, having prevented external conduction, that such water must have passed up internally and been transpired by the plant. She ignores the external films of water which can be readily demonstrated both chemically and microscopically on the freshly gathered plants, especially in the 'pockets' formed by the leaf-bases, and the evaporation of which, when external conduction is prevented, may well have constituted a considerable proportion of the total and relatively small loss which she demonstrates, especially when the leafy shoots were confined in a small chamber through which air was circulating continuously for only a brief space of hours.

The occurrence of external conduction is doubted by Blaikley where the water level falls below the soil surface and 'there is little suggestion of external capillary rise'. It should be pointed out that the plants used in the author's experiments were obtained from a situation which had a well-drained soil, on the surface of which no water visible to the eye accumulated, though it was covered by a certain amount of vegetable debris which felt moist to the touch. In spite of the absence of visible soil water, the plants when gathered had almost invariably quite noticeable droplets of water in the leaf-axils, and, in view of the fact that the surrounding grasses, &c., were quite free from such drops, the only inference which could be drawn was that the water had risen externally and was not the result of precipitation. In all experiments in which a coloured liquid is used, or liquids giving a colour reaction, there is not the slightest doubt as to this rapid upward travel over the external surface, for it can be readily seen in many cases with the naked eye, and certainly by the aid of the reading microscope. This external film of moisture would be absolutely comparable with that which, though invisible to the eye, must evidently occur on the surface of Liverworts and Fern prothallia, since fertilization by motile sperms, requiring water for transit, occurs. Indeed, unless insect fertilization is postulated for all mosses, the occurrence of external water is absolutely necessary in all cases.

On reading Blaikley's work in which she stresses the satisfactory nature of eosin as an indicator of internal rise, the author carried out a very large number of additional tests with this reagent, and found that the reaction depends upon the state of differentiation of the internal tissues, which is extremely variable. Where little lignification occurs the eosin is easily seen; but where the lignification is marked in the central and hypodermal tissues, the detection of the presence of a small amount of eosin is practically impossible on account of the coloration of the tissues, so that the end points of ascent or descent, and the points of entry of the liquid are impossible of determination. It is only where the eosin has accumulated to such an extent as practically to flood the tissue concerned that its presence can be detected with certainty in a coloured tissue; and in any case, Blaikley's method of examining the cut surface at various levels for the presence of eosin in the central strand would not decide the question as to whether the liquid was entering at the base of the stem and rising upwards, or whether it was penetrating in small quantities, practically invisible to the eye or lens, at the points of insertion of the numerous leaves, diffusing into the central strand, accumulating there in larger and therefore more visible amount and moving upwards or downwards in that tissue.

In view of Blaikley's results, which are to a very large extent at variance with those of the author, a very large number of the experiments which had already been carried out with mosses other than *Polytrichum* have been repeated, but invariably with results absolutely consistent with those reported below.

In the present investigation similar methods were used to those described for *Polytrichum*. In each case the external conduction of water was measured, both the rate of ascent and the amount of water conducted being investigated. These figures are considered in relation to the habit of the plant, while the internal conducting capacity of the moss is considered in relation to the development and internal differentiation of the stem and leaf.

Of the mosses studied five species, *Brachythecium rutabulum*, *Philonotis fontana*, *Hypnum cuspidatum*, *Aulaacomnium palustre*, and *Campylopus brevipilus* are typical of those growing in this type of habitat. *Brachythecium rutabulum* is found growing most abundantly in and around waterfalls, whilst *Philonotis fontana*, *H. cuspidatum*, *A. palustre*, and *C. brevipilus* are found in bogs.

#### 1. *Brachythecium rutabulum*.

The material investigated was obtained from the excessively damp environments of waterfalls, where the supply of liquid water was abundant and where the presence of overhanging vegetation prevented rapid evapora-

tion, so maintaining a saturated atmosphere. The moss was found attached to stones and branches of trees in the immediate vicinity of the fall, and was so abundant that it completely covered the substratum, the individual plants imbricating or overlapping each other. The stems are small and weak so that the plants are almost always prostrate, and are branched. The leaves too are small, dark green in colour, very slightly decurrent, and sparsely placed on the stem from which they diverge at an angle of about  $40^\circ$  (Fig. 1).

It was obvious that in so damp an environment a deposit of atmospheric moisture over the whole external surface of the plant was certain, but in the light of the present work it was interesting to investigate the possibility of this moss being able to conduct sufficient water from the substratum should the environment become drier. Moreover, some plants of the species were discovered in rather drier situations slightly removed from the fall, and it seemed possible that their presence in these situations could be accounted for by their power of conducting water.

An investigation was first of all carried out to determine whether any external conduction was possible and, if so, the rate at which it would take place.

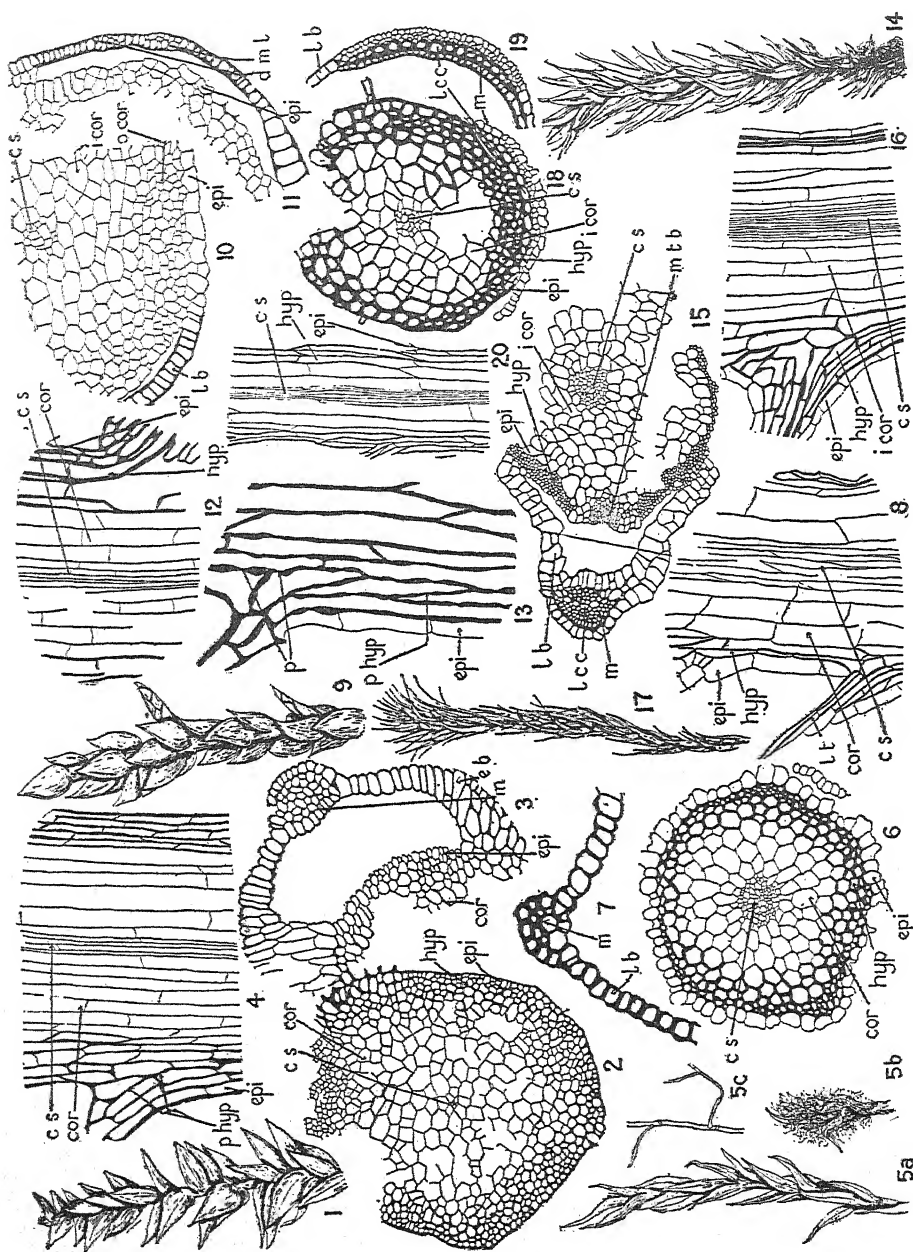
#### *Rate of external conduction.*

The methods used for this investigation were similar to those already described for *P. commune*. Plants whose cut stems had been blocked with paraffin wax were thoroughly washed and air-dried, and being too small to fit into stands as described for *Polytrichum*, were suspended from a line by pieces of fine wire so that their bases were submerged for about 0.5 cm. in dishes containing respectively 0.5 per cent. solutions of gentian violet and dialysed iron, whilst others whose external surface had been dusted over with starch-powder coated with phenolphthalein were submerged for 0.5 cm. in a very dilute solution of sulphuric acid. At regular intervals readings were taken with the reading microscope and two representative results for each of these solutions are given in Table I.

TABLE I.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	3.23	1.56	1.56	1.57	1.57	1.57	1.6
2. "	3.52	1.79	1.8	1.81	1.81	1.88	1.98
3. Iron	2.39	0.82	0.92	1.0	1.05	1.1	1.54
4. "	2.1	0.68	0.78	0.84	1.25	1.31	1.83
5. (Acid)	3.0	1.49	1.52	1.55	1.57	1.61	1.75
6. "	3.6	1.69	1.79	1.8	1.84	1.86	1.96

The rate of external conduction was also measured by investigating the height to which potassium nitrate had risen on the external surface of



Abbreviations for Text-figures.—*cor.*, cortex; *o.cor.*, outer cortex; *i.cor.*, inner cortex; *t.w.cor.*, thick-walled cortex; *c.s.*, central strand; *epi.*, epidermis; *hyp.*, hypodermis; *p.hyp.*, pitted hypodermis; *l.b.*, leaf-blade; *l.t.*, leaf-trace; *m.*, midrib; *l.c.c.*, large central cells of midrib; *d.m.l.*, double midrib of leaf; *m.t.b.*, meristematic tissue of branch; *p.*, pits.)

FIGS. 1-20.—1. Portion of the gametophyte of *Brachythecium rutabulum* showing the arrangement and divergence of the leaves.  $\times 3.5$ . 2. Transverse section of the stem of *B. rutabulum*.



plants which had been treated as above. At intervals the plants were removed, sections were cut and mounted in diphenylamine in concentrated sulphuric acid and examined microscopically for the characteristic deep blue colour reaction. Typical results obtained from these experiments are given in Table II.

TABLE II.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	3.7	1 hr.	1.7
2.	4.5	2 hrs.	3.0
3.	3.2	3 hrs.	2.0
4.	4.5	6 hrs.	3.0
5.	3.5	24 hrs.	3.2

As might be expected, the figures in this table show a more rapid rate of external conduction than do those in Table I, for minute amounts of liquid which can not be seen by the aid of the reading microscope can be detected chemically. Therefore, in reporting the results with every subsequent moss examined, a similar procedure will be adopted; the figures for the materials conducted externally, visible to the eye, being aggregated in one table, and those for the rise of potassium nitrate, which can only be detected by cutting and examining chemically, in another table, though it must be emphasized that this potassium nitrate had risen over the external surface and penetrated from the upper parts of the stem downwards, and had not been conducted internally, for no colour reaction could be obtained in the lower regions until after the upper and apical regions had shown it.

It is evident from Tables I and II that there is no very rapid conduction of water externally and that, though in a dry atmosphere external conduction would supply the lower parts of the plant with water, such conduction is far too slow and limited a process to maintain the plant in a healthy condition.

× 230. 3. Transverse section of the leaf of *B. rutabulum*. × 230. 4. Longitudinal section of the stem of *B. rutabulum*. × 270. 5 (a) Portion of the gametophyte of *Philonotis fontana* showing the arrangement and divergence of the leaves. × 6. (b) Basal portion of the stem of *P. fontana* clothed with tomentum. × 6. (c) Portion of the tomentum. × 137. 6. Transverse section of the stem of *P. fontana* showing thin-walled epidermis and thickened hypodermis. × 270. 7. Transverse section of the leaf of *P. fontana*. × 330. 8. Longitudinal section of the stem of *P. fontana*. × 170. 9. Portion of the gametophyte of *Hypnum cuspidatum* showing the arrangement and divergence of the leaves. × 3.5. 10. Transverse section of a young stem of *H. cuspidatum* showing the thin-walled peripheral tissues and central strand. × 86. 11. Transverse section of the leaf of *H. cuspidatum* taken near its point of insertion on the stem and showing the double midrib. × 81. 12. Longitudinal section of the stem of *H. cuspidatum*. × 76. 13. Longitudinal section of the peripheral tissues of the stem of *H. cuspidatum* showing the thin-walled epidermis and pitted hypodermis. × 240. 14. Portion of the gametophyte of *Aulaacomnium palustre* showing the arrangement and divergence of the leaves. × 3.5. 15. Transverse section of the stem and leaf of *A. palustre*. × 70. 16. Longitudinal section of the stem of *A. palustre*. × 74. 17. Portion of the gametophyte of *Campylopus brevipilus* showing the arrangement and form of the leaves. × 2.6. 18. Transverse section of the stem of *C. brevipilus*. × 128. 19. Transverse section of the leaf of *C. brevipilus*. × 128. 20. Longitudinal section of the stem of *C. brevipilus*. × 66.

*Amount of external conduction.*

Having determined that external conduction does take place at a slow rate, experiments were carried out to determine the amount of water so conducted. The cut stems of plants were again blocked with wax, washed and air-dried, and suspended so that their bases dipped into dishes containing a 0.5 per cent. solution of sodium chloride. These plants were left for intervals of one and five days respectively, for experience showed that these periods gave most satisfactory results. At the end of these intervals the mosses were thoroughly washed and the solution obtained from the washing titrated with a centinormal solution of silver nitrate, potassium chromate being used as an indicator. The following table is typical of the results obtained.

TABLE III.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	3.8	1 day	0.076
2.	2.9	"	0.07
3.	2.2	5 days	0.468
4.	2.4	"	0.175

The results of these experiments confirm the conclusion already drawn that the external conduction of water in these plants is slow and small in amount, and that in no case can it be considered sufficient, in itself, to supply the needs of the plant.

*Rate of internal conduction.*

The leaves were carefully shaved from a length of 0.8 cm. at the base of the stem of these plants and the wounds were painted with collodion. The plants were then suspended as described above so that the basal regions dipped into dishes containing 0.5 per cent. solution of potassium nitrate. At intervals the plants were removed, washed in running water, and sections cut at varying levels on the stem. The sections were mounted in diphenylamine in concentrated sulphuric acid and examined immediately for the blue colour which would indicate that potassium nitrate was present in the tissues.

Other plants treated as above were suspended in bowls of lithium sulphate. After varying intervals the plants were removed, cut into pieces 0.5 cm. long and incinerated in a bunsen flame. The flame was examined spectroscopically for the lithium line. Typical results of both these experiments are given in Table IV.

The results given in Table IV show that the rate of internal conduction is very slow, suggesting that there is no path by which water is conducted

rapidly up the stem, and that the only means by which water is conducted internally is by the slow process of diffusion. This conclusion was verified by examination of the internal structure of the stem.

TABLE IV.

Plant number.	Time.	Readings for potassium nitrate in cm.			Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	18 hrs.	5.0	0.6	0.3	3.2	0.4	0.6
2.	"	3.2	0.6	1.0	4.6	0.5	0.5
3.	24 hrs.	4.0	0.5	0.9	3.5	0.4	0.7
4.	"	3.5	0.4	1.2	2.5	0.5	1.2

*Internal anatomy of the stem and leaf of Brachythecium rutabulum.*

Serial sections of material fixed in Bouin's fluid and embedded in paraffin wax were cut and stained in 50 per cent. alcoholic safranin and Delafield's haematoxylin. Transverse sections of the stem near the apex of the plant showed (Fig. 2) a thin-walled epidermis and outer cortex of small cells, followed by a large-celled, thin-walled inner cortex, which enclosed a central strand of from two to fifteen small thin-walled cells.

Sections of the stem further removed from the apex showed that the epidermis quickly becomes thickened and that this is followed by the thickening of the cells of the outer cortex to form a hypodermis of four or five layers of cells.

The cells of the leaf lamina are continuous with the epidermis of the stem, whilst the midrib of the leaf consists of about fifty cells in transverse section and terminates in the outer cortex or hypodermis (Fig. 3).

Longitudinal sections of the stem (Fig. 4) revealed the mature epidermis and hypodermis to consist of long, narrow cells markedly pitted on all walls, in contrast with the inner cortex of larger and thinner-walled cells; while the central strand was composed of long, narrow, unthickened cells with few cross walls.

*Entry of materials and path of internal conduction.*

Experiments were carried out with complete plants which were dipped into bowls of a 0.5 per cent. solution of potassium nitrate, so allowing both external and internal conduction to take place. Sections of the plants were cut at intervals and mounted in diphenylamine in concentrated sulphuric acid, when a blue coloration was obtained in all tissues into which the nitrate had penetrated. From these experiments it was seen that, although external conduction is so very slow, yet it is more rapid than internal conduction, for colour reactions were obtained in cells of the outer cortex before they were obtained in the inner cortex and central strand. The

experiments described above and recorded in Table IV show that the rate of rise internally is too slow to account for this penetration of potassium nitrate into the outer cortex. It can, therefore, be concluded that whereas this moss normally grows in very wet situations, so that water can be deposited all over its external surface, yet the arrangement of leaves on the stem, with their slightly decurrent leaf-bases, is such as to form channels which will retain capillary films of water. The arrangement of the leaves, however, diverging, as they do, rather sharply from the stem, does not provide continuous narrow channels between leaf and stem, so that the capillary films are restricted to the neighbourhood of the leaf-bases, and in these experiments confined to the lower regions of the stem. The externally conducted water diffuses in through the epidermis and outer cortex whilst water absorbed by the base of the plant is conducted internally through the thin-walled central tissue and inner cortex at a very slow rate.

## 2. *Philonotis fontana*.

In its typical form this species is found in great abundance in the wetter regions of bogs, especially where there is a slow running movement of the surface water. Its habitat is therefore always a wet one, the lower third of the stem being usually submerged in water whilst the atmosphere surrounding the rest of the stem is moisture-laden to such an extent that beads of water are often found on the leaves of the moss. The plants grow in tufts, the stems of which are free from one another in the upper regions but closely interwoven below with tomentum. Each plant is slender, varying from two to twelve centimetres in length and usually producing several branches at the same level on the stem. The leaves are ovate-lanceolate, arranged at closer intervals than in *Brachythecium rutabulum*, and again diverge widely from the stem (Fig. 5).

Although the plant normally grows in a moisture-laden atmosphere, there are periods when the air becomes relatively dry and the chief water supply is then confined to the liquid water below. Therefore, if the higher parts of the stem are to be supplied with water some conduction is necessary.

### *Rate of external conduction.*

The extent to which this took place was investigated by measuring the rate of ascent of gentian violet, dialysed iron, and potassium nitrate according to the methods described above. Representative results in each case are summarized in Tables V and VI.

It is evident from these tables that under drier conditions some water is conducted over the external surface, but that the rate of this conduction is slow, and in no case can it be deemed sufficient to supply the plant with all its water requirements.

TABLE V.

Plant number.	Length of plant in cm.	Eternal rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. ( <i>G. violet</i> )	2.84	0.62	0.72	0.79	0.8	0.8	0.88
2. "	2.56	0.3	0.33	0.37	0.37	0.4	0.48
3. ( <i>Iron</i> )	2.07	0.32	0.32	0.32	0.34	0.37	0.51
4. "	2.11	0.9	0.99	0.99	1.0	1.03	1.11
5. ( <i>Acid</i> )	4.69	0.54	0.57	0.59	0.61	0.66	0.75
6. "	3.21	0.19	0.21	0.24	0.24	0.24	0.24

TABLE VI.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	4.5	1 hr.	1.6
2.	4.5	2 hrs.	2.5
3.	6.2	3 hrs.	1.7
4.	5.2	6 hrs.	2.8
5.	4.8	24 hrs.	3.5

*Amount of external conduction:*

An estimation of the amount of the salt solution conducted externally when the plants were treated as described for *B. rutabulum* gave the following typical results.

TABLE VII.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	1.8	1 day	0.17
2.	3.0	"	0.26
3.	2.5	5 days	0.76
4.	2.4	"	0.53

Since the largest amount of salt solution which had passed up over the external surface of the plant in five days was 0.76 c.c., it must be concluded that external conduction will not suffice to keep this plant in its normal saturated condition.

*Rate of internal conduction.*

Investigation of the rate of internal conduction of dilute solutions of potassium nitrate and lithium sulphate was made and typical results are reported in Table VIII.

It is again evident that internal conduction is very slow, and in no case is it sufficient to supply water to the tip of the plant, although there is a decided increase in the rate of internal conduction as compared with that of *B. rutabulum*.

TABLE VIII.

Plant number.	Time.	Readings for potassium nitrate in cm.			Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	1 hr.	3.0	0.3	0.2	4.1	0.7	0.5
2.	2 hrs.	4.5	0.5	0.1	3.6	1.0	0.8
3.	3 hrs.	4.2	0.6	0.6	4.2	1.0	0.9
4.	4 hrs.	4.5	0.9	0.9	1.9	0.5	1.0
5.	18 hrs.	4.5	0.5	3.0	5.0	0.4	1.6
6.	24 hrs.	4.0	0.3	1.6	3.8	0.6	1.8

*Internal anatomy of the stem and leaf of Philonotis fontana.*

An examination of the internal structure of the stem and leaf of *P. fontana* was made in order to determine whether anything approaching a conducting tissue is present.

Serial transverse sections of the stem showed (Figs. 6 and 7) a thin-walled, large-celled epidermis, followed by an outer cortex of thin-walled cells which become very thick-walled lower down on the stem, and in which the midrib of the leaf terminates; an inner cortex of large parenchymatous and collenchymatous cells, enclosing a thin-walled central strand of small parenchymatous cells occupying about one-sixth of the diameter of the stem.

Longitudinal sections of the stem (Fig. 8) showed the central strand to be composed of elongated parenchymatous cells with oblique walls, the cortex to consist of larger elongated cells with transverse walls, whilst the sclerenchymatous cells of the hypodermis appeared to contain relatively few pits.

*Entry of materials and path of internal conduction.*

Experiments with complete plants whose bases dipped into solutions of potassium nitrate were carried out, in the manner described for *B. rutabulum*, in order to determine the path of entry of solutions. Accurate results were extremely difficult to obtain owing to the smallness of the stem and also to the transient nature of the colour reaction. Sufficient data were obtained, however, to justify the conclusions that (1) the small central tissue was chiefly concerned with internal conduction, though the limited length of the cells curtailed its rate; (2) absorption of the externally conducted liquid was carried out by the thin-walled living cells of the epidermis, which are put into communication with the cortex by the extremely limited number of pits on the walls of the hypodermis.

Surveying all the data available the conclusion to be drawn is that under normal conditions *P. fontana* is surrounded by a saturated atmo-

sphere and thus has no need to conduct water ; but that, in times of greater drought, conduction does take place both internally and externally to some small extent. Internally, there is a greater differentiation of the central strand than is found in *B. rutabulum*. This strand in *P. fontana* consists of a much larger number of elongated cells than in the former case, whilst externally the hypodermis, which is thick-walled, is bounded by a thin-walled epidermis of large cells by which water conducted over the exterior of the plant can be absorbed osmotically and diffused from cell to cell.

### 3. *Hypnum cuspidatum*.

The third moss of this wet environment is found growing very abundantly in a habitat similar to that of *P. fontana*, but is confined chiefly to regions of still water, whereas *P. fontana* is most common in small streams of running water. Like this moss, the lower third of the stem is submerged, but here the individual plants are not tufted but are more or less separate. They are often inclined from the perpendicular and are always densely branched. The plants vary from three to twelve centimetres in length, but are sometimes longer and then they assume a more creeping habit. The stems are round, firm, and crowded with small, slightly sheathing leaves which become convolute at the apex, giving the species its characteristic appearance (Fig. 9).

Experimental work on this moss was carried out along lines similar to those described for the previous species.

#### *Rate of external conduction.*

The rates of external conduction of gentian violet, sulphuric acid, dialysed iron, and potassium nitrate are given in Tables IX and X.

TABLE IX.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	3.6	1.32	1.81	1.89	1.89	1.98	2.06
2. "	4.58	1.45	1.52	1.59	1.65	1.79	1.89
3. (Iron)	3.3	1.73	2.42	2.42	2.46	2.46	2.5
4. "	3.16	2.32	2.4	2.45	2.46	2.46	2.5
5. (Acid)	4.94	1.3	1.41	1.68	1.84	2.23	2.46
6. "	4.32	2.1	2.15	2.63	2.94	3.9	4.32

TABLE X.

Plant number.	Length of plant in cm.	Time.	External rise of KNO <sub>3</sub> in cm.
1.	4.5	1 hr.	3.2
2.	6.5	2 hrs.	3.0
3.	6.0	3 hrs.	4.0
4.	7.1	6 hrs.	4.8
5.	8.0	24 hrs.	6.1

It can be concluded that there is a more rapid conduction of water in this moss than in either *B. rutabulum* or *P. fontana*, and that in some cases, especially where the stems are densely branched, external conduction is sufficient to supply water to the tips of the moss. Moreover, as has already been pointed out, these plants are often inclined from the vertical, and this facilitates the external rise of liquids.

*Amount of external conduction.*

Experiments measuring the amount of liquid conducted externally were carried out and typical results are tabulated as under.

TABLE XI.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	4.5	1 day	0.58
2.	5.5	"	0.32
3.	5.0	5 days	2.75
4.	4.1	"	2.75

External conduction in this moss is therefore responsible for the rise of a considerable amount of liquid, as much as 2.75 c.c. of liquid being carried up in five days. In view of the size of the plant and the possibility of the absorption of part of the conducted salt into the interior, which would mean that the figure given is an underestimate of the total amount, this is obviously a very appreciable quantity.

*Rate of internal conduction.*

The existence of an external conducting capacity which is fairly efficient in view of the moist environment does not necessarily eliminate the possibility of internal conduction, and so further experiments were carried out in order to elucidate this point. Plants were treated as described above and suspended so that the basal portions of the stems dipped into bowls containing respectively 0.5 per cent. solutions of potassium nitrate and lithium sulphate, and the heights to which these solutions had risen were determined as for *B. rutabulum*. Typical results obtained are given in Table XII.

Results obtained are by no means uniform, although in all cases the rate of internal conduction is very much slower than the rate of external conduction.

An examination was then made to decide whether any variation in internal differentiation could be definitely correlated with this fluctuation in the rate of internal conduction of liquids.



TABLE XII.

Plant number.	Time.	Readings for potassium nitrate in cm.			Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	1 hr.	3.5	0.3	0.6	2.6	0.5	0.5
2.	"	1.5	0.5	0.2	2.5	0.7	0.9
3.	2 hrs.	4.5	0.5	1.2	4.5	0.5	0.8
4.	"	6.0	0.5	0.2	6.2	0.7	0.2
5.	3 hrs.	7.5	0.4	0.2	5.1	0.9	0.4
6.	"	5.0	0.5	0.3	5.5	1.0	0.9
7.	18 hrs.	6.5	0.7	1.4	7.1	0.7	0.6
8.	"	7.5	0.5	1.4	6.2	0.9	1.5
9.	24 hrs.	4.1	0.4	1.7	6.0	0.3	0.9
10.	"	6.5	0.5	1.5	3.9	0.5	1.7

*Internal anatomy of the stem and leaf of Hypnum cuspidatum.*

Transverse sections of young stems and sections cut near the apices of older stems showed a large-celled, thin-walled epidermis, an outer cortex of thin-walled smaller cells, an inner cortex of thin-walled large cells, and a central strand of thin-walled small cells occupying about one-tenth of the diameter of the stem (Fig. 10).

Sections of older stems showed that, as in *P. fontana*, the epidermis always remains thin-walled whilst the outer cortex becomes extremely thickened to form a hypodermis of from six to ten layers. The inner cortex and central strand always remain thin-walled.

The leaf is continuous with the epidermis, the midrib where present terminating in the hypodermis. The midrib consists of a double strand, each comprising about twelve cells thickened in a similar way to the hypodermis of the stem. The tissue between these two strands consists of a single layer of about three thinner-walled cells comparable with those of the lamina (Fig. 11).

An examination of longitudinal sections of the stem showed that the hypodermis is very densely pitted, the number of pits increasing towards the inner side of the hypodermis. These pits are conspicuous even in the outer thicker cellulose walls of the cortex. The cortex is composed of large, elongated cells whilst the central smaller cells are very elongated with few transverse walls (Figs. 12 and 13).

*Entry of materials and path of internal conduction.*

Adopting the same methods as before, it was found that in young stems internal conduction was appreciable while in older stems it was negligible. In the young stems, the hypodermis is relatively thin-walled and so the epidermis is in direct continuity with the cortex. There is,

therefore, an easy passage of water and dissolved elaborated food materials through to the central cells, thus probably increasing the osmotic concentration of these materials in the long, narrow cells of the central tissue and facilitating the passage of water through them. In older stems, however, the hypodermis is thick-walled and, although pitted, does not allow the rapid transference of materials to the central tissue.

The epidermis, however, which is in contact with the photosynthetic leaves, retains the power of absorbing water osmotically and so sends it on its journey from cell to cell of this outer layer to the tip of the plant where it penetrates into the interior. This can be regarded as, in a sense, a physiological means of external conduction, but it is very greatly supplemented by the purely physical method of capillarity facilitated by the dense and compact arrangement of the leaves on the stem. Experiments carried out with complete plants showed that, whatever the *means* of external conduction, a fairly rapid *rate* occurred, and where the water so conducted reached the tip of the plant penetration was also rapid. In cases in which the liquid was not conducted externally to the tip, the rate of penetration, as determined by potassium nitrate solutions, was very slow and, even in one and the same plant, sections showed complete coloration on treatment with diphenylamine, indicating penetration, when taken from the upper parts of the stem, many hours before such coloration was visible in the cortex of similar sections taken from the lower parts of the stem. For instance, in a plant 5.5 cm. long, at the end of twenty-four hours there was complete coloration from the tip to 1.2 cm. from the base, while below this region there was no coloration in the stem except in the central strand, which showed coloration for 0.8 cm. from the base of the plant.

Thus it can be concluded that in *H. cuspidatum* external conduction can, and probably does take place, the water entering the plant both through the hypodermis and through the unthickened apex, but that the more rapid entry takes place in the latter case. Moreover, the young stems of this moss have the power of conducting water internally, and so can partly supply their needs in this way.

#### 4. *Aulacomnium palustre*.

This large species is found growing in bogs often intermingled with *Sphagnum*. The plants grow in tufts, the individual stems being thick, from one to five inches long, unbranched and covered for three-quarters of their length with a dense mass of tomentum. The leaves are crowded, large, slightly sheathing at the base and taper to very pointed apices (Fig. 14).

*Rate of external conduction.*

An investigation of the rate of external conduction, carried out in the usual way, gave the following typical results:

TABLE XIII.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	4.85	1.46	2.1	2.4	2.9	3.4	3.5
2. "	7.4	3.4	3.75	4.3	4.5	4.5	5.2
3. (Iron)	4.3	2.36	2.7	3.2	3.4	3.9	4.3 (tip)
4. "	8.5	1.75	2.6	2.6	2.7	3.0	4.8
5. (Acid)	7.2	3.26	5.1	5.2	5.4	5.5	6.2
6. "	6.45	6.2	6.4	6.4	6.4	6.4	6.45 (tip)

Plant number.	Length of plant in cm.	Time.	External rise of KNO <sub>3</sub> in cm.
1.	4.7	1 hr.	2.3
2.	4.0	2 hrs.	1.1
3.	5.8	3 hrs.	5.3
4.	5.3	6 hrs.	4.7
5.	5.5	24 hrs.	5.5 (tip)

The rate of external conduction is here more rapid than in any moss so far investigated, and is sufficient in some cases, under laboratory conditions, to supply the tips of the plants with water. This external supply will be even greater in its normal habitat where the tufted habit of the plant, its contact with *Sphagnum* and the presence of tomentum will facilitate the retention of external films of water.

*Amount of external conduction.*

Typical readings of the amount of liquid conducted externally are given in Table XIV.

TABLE XIV.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	5.8	1 day	0.585
2.	4.4	"	0.468
3.	3.9	5 days	2.925
4.	3.6	"	2.048

The above table shows that the amount of solution conducted externally is large and, in comparison with the size of the plants, is the greatest that has so far been recorded.

*Rate of internal conduction.*

Experiments carried out with cut stems over which external conduction had been prevented, as previously described, showed an internal rise of potassium nitrate and lithium sulphate to the extent indicated in Table XV.

TABLE XV.

Plant number.	Time.	Readings for potassium nitrate in cm.			Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	1 hr.	7.0	0.5	0.3	6.8	0.3	0.4
2.	2 hrs.	7.2	0.6	0.7	7.2	0.3	0.3
3.	3 hrs.	8.1	1.1	0.3	6.0	0.2	0.6
4.	6 hrs.	3.8	1.0	1.2	5.7	0.3	0.6
5.	18 hrs.	4.6	0.25	1.6	4.9	0.4	1.5
6.	24 hrs.	7.5	0.4	1.7	5.2	0.3	1.8
7.	3 hrs.	5.6	0.7	1.1	6.4	0.5	3.2

It is obvious that this moss, like the previous forms studied, has only a small capacity for conducting solutions internally.

*Internal anatomy of the leaf and stem of Aulacomnium palustre.*

A typical transverse section of the stem and leaf of this moss (Fig. 15) shows in the stem, the thin-walled epidermis surrounding a several-layered hypodermis of small cells, which is interrupted in the region of a lateral branch; a wide thinner-walled inner cortex and a relatively large central strand. In the leaf, a large-celled, thin-walled lamina is continuous with the epidermis of the stem, and a midrib of a row of large central cells occurs, which in longitudinal section are seen to be continuous with the inner cortex. Surrounding these are a large number of thick-walled, outer cells which terminate in the hypodermis of the stem.

A longitudinal section of the stem (Fig. 16) shows the thin-walled epidermis, the hypodermis which is sparsely pitted, the larger cells of the inner cortex, and the very long and narrow cells of the central strand.

*Entry of materials and path of internal conduction.*

As in *H. cuspidatum* external conduction is much more rapid than internal conduction. This was very clearly seen when complete plants were used; for instance, at the end of one hour potassium nitrate had in some cases reached the apex of the plant externally and penetrated into the outer thin-walled tissue at the tip; below this there was an indication of penetration into the outer hypodermis, but no coloration was visible in the central tissues at the base or higher up the stem. Sections cut at the end of twenty-four hours revealed a gradual passage of potassium nitrate

through the cortex, &c., is only very slightly tortuous, comes to be attached to the conducting strand of the rachis. Inside, the haustorium branches into two forks—one attaches itself to the xylem and the other to the phloem (Figs. 3 and 4).

Thus by this efficient device, the parasite is able to draw its nourishment from both the forms of food material—raw and elaborated—available in the host plant.

It appears that the determining factor in the attack here also—as shown by Parija in the case of angiosperms—is the relation of the osmotic concentration of the parasite to that of the fern hosts. Investigation in this direction is in progress, and I hope to publish the results shortly in a separate communication.

I am very grateful to Professor P. Parija for allowing me to make use of certain conclusions from his unpublished paper, and also for kindly offering me facilities to carry on this work in his laboratory.

CALCUTTA.

T. C. N. SINGH.

**A NEW DEVICE FOR OVERCOMING ELECTRIFICATION IN MICROTOMY.**—Electrification in microtome work is a real problem in a tropical climate like that of India. Only during the winter (from November to March) is it possible to do such work with any success.

It was, therefore, with a view to obviate this handicap of electrification, and consequently facilitate work, even in hot months, that the present investigation was taken up by the author in March 1928, at the Agricultural College, Lyallpore (Punjab). A satisfactory solution was, however, reached only last year. It is presented in the hope that it will prove helpful to some fellow-workers.

Because of its simplicity and cheapness, the Cambridge Rocking Microtome is the only one which is in general use in most of the Indian laboratories in preference to more costly microtomes. Dr. W. J. G. Land's arrangement<sup>1</sup> on the Rotary Microtome is quite successful, but its complication and high price limit its general use, especially in India. So the experiment was tried only with the cheaper Cambridge Rocking Microtome.

At first water-cooled, and then ice-cooled, knives were employed for cutting sections, but it was found that after a few sections the knife became electrified, with the result that it needed recooling. Later, ice blocks were so arranged that they rested on the free ends of the cooled knife, but this measure also did not improve matters. Nevertheless, the application to a cooled knife-edge of drops of cold water from time to time, by means of a soft camel-hair brush, certainly helped to do away with the electrification to some extent as long as the edge-moisture was maintained. It was on this principle of somehow keeping the knife-edge constantly moist with cold water during the operation of the machine that the present apparatus was devised.

*The apparatus.* The apparatus is very simple and, at the same time, within the means of practically every worker. It can be made from canister-sheet, brass or aluminium, by the necessary moulding and soldering, which in any part of India will never cost more than a rupee.

<sup>1</sup> C. J. Chamberlain : Methods in Plant Histology.

The apparatus in its finished form consists roughly of a T-shaped tank (see *text-figures*). The microtome-knife is placed in the horizontal limb (*h*) of the T-tank, and the whole arrangement is then mounted in the slots of the microtome knife-pillars. Lead wedges (*w*) are inserted in the two gaps on either side opposite the

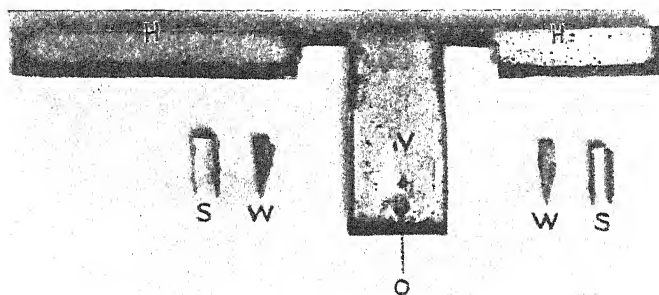


FIG. 1. Photograph of the tank as seen from above.

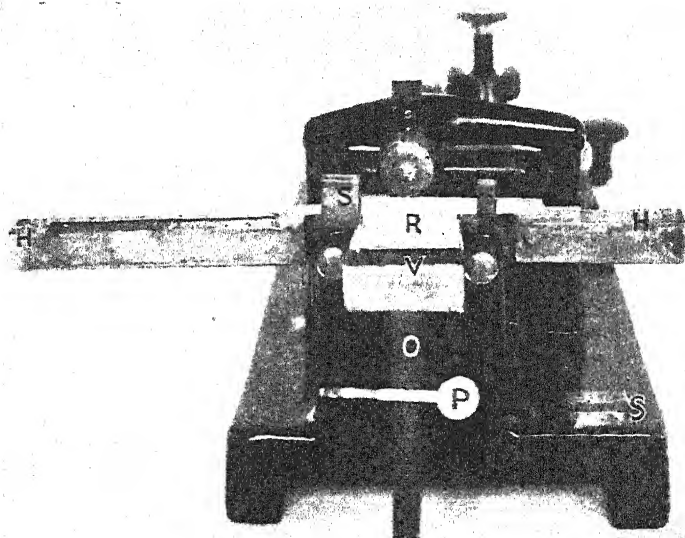


FIG. 2. Photograph of the tank on the microtome: H, horizontal limb; V, forwardly projecting tank; W, wedge made of lead; S, binding strip of the same material as the tank; R, microtome knife; O, outlet for water, with rubber-tubing; P, pinchcock.

pillar-screws, and binding-strips<sup>1</sup> are placed saddle-wise over the wedges before finally screwing up the apparatus to keep it steady while the machine is in use. The tank is then filled with ordinary cold water (preferably from an earthen pitcher), which is allowed to play just very near to the edge of the knife. No previous cooling of the knife, however, is necessary.

The apparatus so adjusted is at once ready for use. The paraffin block with

<sup>1</sup> These binding strips are often unnecessary.

the material embedded in it, is cut in the usual manner without any danger of electrification. The sections as they are cut float in the water contained in the limb (*v*) of the T-tank.

This device entirely prevents the troublesome electrification and sections to any desired thickness may be cut with perfect ease, even in the depth of summer, the ribbon being continuous. The water of the tank, however, should be changed when necessary.

During summer months the apparatus was tested at the Institute of Plant Industry, Indore (Central India), and the Benares Hindu University, Benares (United Provinces), and the results obtained were completely satisfactory.

I take this opportunity of thanking Professor N. K. Tiwary, of the Benares Hindu University, and his students, for kindly giving the apparatus a number of trials.

T. C. N. SINGH.

NAINI TAL (INDIA).

November 25, 1931.

#### A SIMPLE DEVICE FOR REGULATING GAS FLOW.—

In many investigations in plant physiology there is much need for a method of regulating with ease and exactitude the flow of gases and liquids. The one generally adopted, consisting of rubber pressure tubing compressed by means of screw clips, with or without the insertion of a metal wire in the tube, is not altogether satisfactory, as the rate of flow is difficult to control at a required speed, except at very low values. The use of capillary tubes for control of gas flow has been previously tried, e.g., by Boysen Jensen, but no means of varying the rate of flow over a continuous range was suggested.

The simple apparatus here described gives complete control, at any desired speed, of the flow of a gas or fluid. It consists essentially of a capillary of variable length, thus enabling the resistance to flow to be varied at will over a large range. A diagrammatic representation of the apparatus is appended.

It may be constructed from a piece of glass tubing of suitable length and diameter— $\frac{1}{4}$  in. internal diameter has been found convenient. One end of the tube is extended by fusing on a piece of wider glass tubing ( $\frac{3}{8}$  in. has been used), of convenient length, and to this wider tube a side tube is attached as shown in the diagram. Within the main tube moves a plunger consisting either of a solid glass rod or of glass tubing closed at both ends. The resistance of the regulator depends on the accuracy with which the plunger fits the cylinder. For moderate resistances a 'sliding fit' may be obtained by suitably selecting the glass tubing and glass rod. For very high resistances the



plunger should be ground down with emery powder in a similar separate tube, and finally ground to fit the cylinder with very fine emery powder. It is important that all glass blowing should be completed before any attempt is made to grind in the plunger, as otherwise longitudinal cracks in the cylinder invariably develop on heating. A gas-tight joint between plunger and cylinder is established by passing the plunger through a length of pressure tubing at the upper end of the cylinder, the rubber tubing having a diameter slightly less than that of the plunger. Ordinary rubber connexions are unsatisfactory as under negative pressure the rubber tubing tends to bind the plunger and thus makes adjustment difficult. The gas flows in the apparatus between the side tube and the open end of the cylinder—the direction is immaterial.

Regulation of gas flow is effected by moving the plunger up or down the cylinder, thus varying the resistance to flow by alteration of the length of the capillary space between the plunger and the wall of the cylinder.

The completed regulator may be calibrated for known rates of flow and pressure differences, and a chart prepared giving rates of flow for known pressures against the position of the plunger in the cylinder. Such graduations may be permanently attached to the cylinder, and therefore so long as the pressure under which the gas is moving through the regulator is known and maintained, any desired rate of flow may be obtained by appropriate setting of the plunger. The relation between length of resistance and rate of flow is found to be linear over a large range.

The apparatus has been successfully employed in this laboratory for the last two years in regulating the gas flow in respiration experiments, and has many other obvious applications. The writer wishes to thank Mr. J. I. Armstrong of this Institute for the actual construction of the apparatus and for successfully overcoming all the practical difficulties encountered.

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downwards from the tip into all the tissues. This passage downwards, however, is slow and is accompanied by a lateral penetration from the exterior into the inner layers of the hypodermis on the lower parts of the stem, this penetration being most evident in the region of the leaf-bases. Entry into the central strand and cortex of the lower regions of the stem was not evident for three or four days.

Internal upward conduction must, therefore, play only a small part in supplying water to the higher regions of the stem, the necessary supplies being obtained by the ready entry through the tip and downward diffusion of externally conducted water, accompanied by a much slower lateral diffusion inwards of water which has entered slowly through leaf-bases and passed through the sparsely pitted hypodermis.

### 5. *Campylopus brevifolius*.

This fifth and last form studied from the first type of habitat is found growing on moist heaths and the drier regions of a bog. The plants are short and grow in loose tufts, forming conspicuous bright green, silky patches. The stems are slender and easily separable, black in the basal two-thirds and with leaves interruptedly tufted. The leaves are narrow with tapering, slightly-sheathing bases and sharply pointed apices (Fig. 17).

Experimental work on this moss was conducted along lines similar to those described for previous mosses.

#### *Rate of external conduction.*

Typical results of the rate of external conduction are given in Tables XVI and XVII.

TABLE XVI.

Plant number.	Length of plant in cm.	External rise of liquids in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	2.37	1.8	2.0	2.37 (tip)			
2. "	3.07	1.22	1.5	3.07 (tip)			
3. (Iron)	3.18	1.2	1.5	2.1	2.5	2.7	3.18 (tip)
4. "	2.9	1.6	2.7	2.86	2.9 (tip)		
5. (Acid)	2.83	2.83 (tip)					
6. "	3.08	2.4	2.7	3.08 (tip)			

TABLE XVII.

Plant number.	Length of plant in cm.	Time.	External rise of KNO <sub>3</sub> in cm.
1.	4.7	1 hr.	4.0
2.	4.8	2 hrs.	4.8 (tip)
3.	5.7	3 hrs.	5.7 (tip)

Since potassium nitrate had in most cases reached the apex of the

plant in two hours, it is clear that external conduction is exceptionally effective in the case of this rather short moss. It is interesting to note that this species which has the power to conduct water at a rate more rapid than any form so far reported, was found in a habitat, which, although a wet one, is drier than the habitats of the previous forms.

*Amount of external conduction.*

Typical results obtained for the volume of salt solution conducted are given in Table XVIII.

TABLE XVIII.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	2.8	1 day	0.175
2.	3.8	"	0.295
3.	3.1	5 days	0.644
4.	3.5	"	1.17

These apparently small volumes of liquid, when considered in relation to the small size of the plants, are amply sufficient to keep them in healthy condition when water is restricted to their lower regions.

*Rate of internal conduction.*

An examination of the rate of internal conduction gave the following typical results:—

TABLE XIX.

Readings for potassium nitrate in cm.					Readings for lithium sulphate in cm.		
Plant number.	Time.	Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	1 hr.	3.7	0.3	0.1	4.2	0.3	0.1
2.	2 hrs.	3.8	0.5	0.1	3.6	0.5	0.1
3.	3 hrs.	5.8	0.7	0.1	4.1	0.3	0.1
4.	4 hrs.	6.5	0.5	0.2	4.8	0.4	0.2
5.	1 day	3.7	0.7	0.6	2.4	0.4	0.7
6.	3 days	3.9	1.0	1.2	3.8	0.4	1.1

The rate of internal conduction is again very slow and can in no way be deemed sufficient to supply the needs of the plant.

*Internal anatomy of the stem and leaf of *Campylopus brevipilus*.*

Typical sections of an older stem and leaf of this moss (Figs. 18 and 19) show that both the epidermis and hypodermis of the stem are thickened, and that the inner cortex is composed of larger cells with thinner walls. The central strands consist of from five to fifteen small, thin-walled cells.

The hypodermis of the stem is continuous with the midrib of the leaf and consists of three or four layers of thick-walled cells—the centre layer being larger than those on either side. The upper layer of the midrib is thinner walled and is continuous with the lamina and the epidermis of the stem.

In longitudinal section the central strand is seen to consist of very long narrow cells with few transverse walls, and the thickened elongated cells of the hypodermis to be sparsely pitted (Fig. 20).

*Entry of materials and path of internal conduction.*

A comparison of the rates at which liquid is conducted internally and externally in this species shows that here again external conduction is by far the more rapid. Penetration of this externally conducted solution is most rapid at the tip, and all the tissues in this region are flooded for a distance of 0.5 cm. from the tip in twenty-four hours. Entry through the hypodermis is very slow, for no coloration of the inner cortex and central strand was seen in the lower regions of the stem at the end of three days. This can be correlated with the few pits found in the walls of these cells. Conduction upwards through the central strand from the liquid penetrating through the base of complete plants is very small and rarely had liquid risen more than 0.5 cm. in twenty-four hours.

### CONCLUSION.

It can thus be concluded that all the mosses investigated from this first wet type of habitat possess a definite, though variable, capacity for conducting water when environmental conditions are such that only the lower regions of the stem are in contact with the water. The conduction of the liquid is most rapid on the outside of the plant and varies in speed with the arrangement and form of the leaves and the habit of the plant, these characters in themselves varying with the amount of water in the habitat. The plants are also able to conduct some liquid internally, but in all cases this amount is small. The supplies of water for the internal tissues penetrate chiefly at the apex of the stem and pass downwards, though these supplies are augmented by small quantities which enter chiefly in the region of the leaf-bases and spread laterally through the tissues.

The writer's best thanks are extended to Dr. F. A. Mockeridge, Head of the Department of Biology, University College of Swansea, for all advice given and criticisms made during the carrying out of this work; to Mr. W. R. Sherrin, of the British Bryological Society, for aid in the accurate identification of some of the species investigated; and to

Mr. L. Thomas, of the Department of Biology, University College of Swansea, for assistance in the photographing of drawings.

# SUMMARY.

1. Five species of Musci were selected from very wet habitats and their method of obtaining water investigated.

2. Various methods were adopted in order to measure the rate and amount of conduction of water, both over the external surface and through the internal tissues, and the results of these are recorded.

3. The external morphology and habit of the plants were studied in connexion with their capacity to conduct water externally, and it was found that there is a definite correlation between the two.

4. The internal structure of the stems and leaves of the species under investigation was examined, and the paths of internal conduction and of entry into the stem tissues of the externally conducted liquids were determined.

5. In all forms the amount of water conducted over the external surface exceeded that conducted internally.

6. The water conducted externally ascended in the form of capillary films between the leaves and the stem, and was absorbed by the unthickened cells at the apex of the stem and in the leaves and branches, and diffused through the internal tissues in a lateral and downward, rather than in an upward, direction.

7. It was found that the water ascending internally travelled through the narrow, elongated, thin-walled cells of the central strand.

8. It was found that, in general, the power of the plant to conduct water both externally and internally diminished as the moisture content of the habitat increased, presumably owing to the fact that the moister the habitat the more water to supply its needs would be deposited from the humid atmosphere over the surface of the plant.

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## NOTES.

**CUSCUTA AS A PARASITE ON A FERN.**—While in the Naini Tal Hills (North-Western Himalayas), from June to December, 1931, I was surprised to see the plurivorous *Cuscuta reflexa* Roxb. growing as a parasite on almost all the angiospermous plants—ranging from high trees to prostrate grasses—but with the complete exclusion of cryptogams and gymnosperms. Even in a mixed formation of all these plants infested with *Cuscuta*, the angiosperms alone were the target of attack. In several cases I observed the coils of the parasite actually twined round the leaves of ferns or mosses, but these were always left untouched. This selective nature of the parasitism appeared to be interesting, so it was decided to repeat artificially an experiment on different mosses and ferns growing in their natural habitat. The results obtained have proved to be interesting, and appear to deserve a record.

Perusal of the careful work of Pierce, Harris, Thomson, Moss, Parija,<sup>1</sup> and a few others, has shown that none of them seem to have observed the attack of the parasite on cryptogamic and gymnospermous hosts. All these authors have studied the parasite in relation to angiospermous hosts only.

**Experiment:** Pieces of *Cuscuta* torn off from their phanerogamic hosts were trained round the aerial portions of several mosses and ferns growing *in situ*. This was done in order to expedite attack. A control angiospermous plant was similarly treated. Observations were taken every alternate day.

**Result:** As a result of these experiments it was found that the parasite thrived quite luxuriantly on the control, and the attack, as anticipated, was very severe, while on the other hand the fragments of the parasite on the experimental plants dried up completely during the course of ten days without displaying any evidence of attack except on themselves. There was, however, one case of a fern, *Athyrium pectinatum* Wall., which alone—out of two dozen of the fern species experimented upon—fell a prey to the attack of the parasite.

The first signs of attack could easily be seen by a little tighter coiling of the parasite round the rachis of the fern but not round the petiole, perhaps because of the tough nature of its outer skin. The direction of the coil was a left-handed one,

<sup>1</sup> HARRIS, J. A.: The Tissue Fluids of *Cuscuta*. Bull. Torrey Bot. Club., li. 1924.

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i.e. in a clockwise direction (Fig. 1). In this regard my observation differs from that of Pierce (1894) who says that 'so far as I have been able to see, they twine always in the reverse direction of the hands of the watch'. However, at the point



FIG. 1.

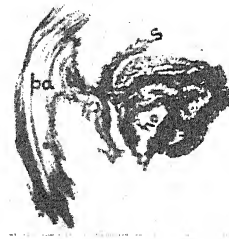


FIG. 2.

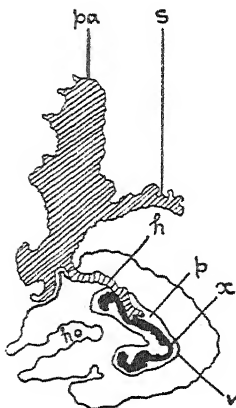


FIG. 3.



FIG. 4.

(ho. = host; pa. = parasite; s. = saddle-shaped structure; h. = haustorium; v. = vascular strand; x. = xylem; p. = phloem.) FIG. 1. Portion of a frond of *Athyrium pectinatum*, Wall. with *Cuscuta reflexa*, Roxb., wound tenaciously round the rachis of the fern in a clock-wise manner.  $\times 1$ . FIG. 2. A section through the rachis with the parasite attached to it. The haustorium is seen to be arising centrally from the saddle-shaped structure (s.) and penetrating the rachis of the host.  $\times 22$ . FIG. 3. Same as Fig. 2; showing the course of the haustorium through the cortex down to the vascular strand (v.). The parasite (in hatching) and the forking of its haustorium is clearly seen.  $\times 32$ . FIG. 4. A portion of Fig. 3 magnified to show the forking of the haustorium more clearly.  $\times$  abt. 150.

of contact the stem of the parasite was observed to become thickened like a cushion. This, in section, looks like a saddle-shaped structure adpressed to the surface of the rachis (Fig. 2). From the central region of this structure arises a haustorium which pierces through the rather hard epidermis, subsequently penetrating the cortex, endodermis, and the pericycle of the host. Thus the haustorium, whose course

# The Influence of Length of Day on the Response of Plants to Boron.

BY

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With Plates XVIII and XIX and one Figure in the Text.

## CONTENTS.

	PAGE
I. INTRODUCTION . . . . .	430
II. METHODS . . . . .	431
A. Light Control . . . . .	431
B. Temperature Conditions . . . . .	432
C. Nutritive Conditions . . . . .	432
III. EXPERIMENTAL DATA . . . . .	434
A. <i>Vicia Faba</i> . . . . .	434
1. Development under short day conditions . . . . .	434
2. Dry weight and nitrogen content . . . . .	436
(a) Influence of the length of day on the effect of boron deficiency . . . . .	436
(b) Influence of boron deficiency on the effect produced by a shortened day . . . . .	436
B. <i>Phaseolus multiflorus</i> . . . . .	438
1. Development under short day conditions . . . . .	438
2. Dry weight and nitrogen content . . . . .	439
(a) Influence of the length of day on the effect of boron deficiency . . . . .	439
(b) Influence of boron deficiency on the effect produced by a shortened day . . . . .	439
C. <i>Hordeum vulgare</i> . . . . .	441
1. Development under short day conditions . . . . .	441
2. Tiller formation in the presence and absence of boron under full and short day conditions . . . . .	443
3. Dry weight and nitrogen content . . . . .	444
(a) Influence of the length of day on the effect of boron deficiency . . . . .	444
(b) Influence of boron deficiency on the effect produced by a shortened day . . . . .	446
4. Response to boron . . . . .	447
D. <i>Glycine hispida</i> . . . . .	449
1. Development under short day conditions . . . . .	449
(a) Variety Biloxi . . . . .	449
(b) Variety Mandarin . . . . .	450

	PAGE
2. Dry weight and nitrogen content . . . . .	450
(a) Influence of the length of day on the effect of boron deficiency . . . . .	450
(b) Influence of boron deficiency on the effect produced by a shortened day . . . . .	452
E. <i>Pisum sativum</i> . . . . .	452
1. Development under short day conditions . . . . .	452
2. Dry weight and nitrogen content . . . . .	453
(a) Influence of the length of day on the effect of boron deficiency . . . . .	453
(b) Influence of boron deficiency on the effect produced by a shortened day . . . . .	453
IV. GENERAL DISCUSSION . . . . .	454
V. SUMMARY . . . . .	455

## I. INTRODUCTION.

EARLIER work in this laboratory on the essential nature of boron in plant nutrition (2, 18), which has since been corroborated and extended by a number of other workers, has shown that although the whole plant eventually dies if deprived of this element, it is the meristematic tissues which are primarily affected by its absence. The symptoms of a deficiency of boron appear first at the growing apices of shoot and root, flowers are rarely if ever produced and, in the case of leguminous plants, nodules fail to develop normally (1). Beyond this, it has not yet been possible to assign any definite function to the element, in spite of a fairly thorough investigation of the matter.

Certain points, however, arose in the course of this earlier work which appeared of sufficient interest to merit further investigation, one of which is the subject of the present paper.

It had been noticed constantly that when plants were grown without boron in the spring or autumn a longer time elapsed<sup>1</sup> before the deficiency symptoms appeared, than if they were grown during the summer. Two obvious reasons for this at once suggested themselves, viz. the lower temperatures and the poorer light conditions in the spring and autumn compared with the summer. The problem, however, was not so simple as it looked at first, for this delay in the appearance of deficiency symptoms in the plants grown without boron was accompanied by a delay in the appearance of the flowers on the plants supplied with it. Further inquiry was therefore needed to see if this indicated either one, a special relationship between the function of boron and flower formation (although this element was already known to play a part in vegetative growth as well), and/or two, that the need of the plant for boron was affected by changes in external conditions such as light or temperature.

Several authors have shown that flowering may be controlled by the

<sup>1</sup> The reason why any time elapses before differences appear between the plants grown with and without boron is no doubt due to the presence of the small quantity of boron in the most seeds which proves sufficient for the needs of the plant during the early stages of growth.



temperature to which the plants are exposed either during the night only (5), or during both day and night (9), or even during the process of germination only (13). Others again, of whom perhaps Garner and Allard's names are best known, have demonstrated that the flowering of most plants depends largely on the number of hours of daylight to which they are exposed. Certain plants, usually termed 'short day' plants, only produce flowers if the number of consecutive light hours are few enough, whereas others known as 'long day' plants, only form reproductive organs if exposed to a sufficiently long period of light. A 12-hour day is taken as the dividing line between these two categories, and photoperiodism is the term applied to the response of the plant to length of day conditions.

That nutritive conditions may control the response of the plant to changes in the length of day has been shown by Maximov (12) who found that although barley grown in water culture with full nutrients under a short day failed to form reproductive organs, if nitrogen were omitted from the solution, ears were produced. There seems no reason, therefore, why the reverse effect should not also hold and the nutritive requirements of the plant be modified under altered light conditions. It will be evident that these phenomena afford a ready means of testing out such problems as are referred to above, for by comparing the behaviour of plants grown with and without a supply of boron under a length of day which prevents or at any rate retards and limits flowering, but allows a good vegetative growth, with those grown under ordinary daylight, an answer may be obtained simultaneously to both the questions already brought forward. The temperature conditions for the two sets of plants must of course be similar. Water-culture experiments were accordingly carried out on these lines with a number of plants which were known to require boron under normal light conditions, and might be expected to respond to alterations in the length of day to which they were exposed.

## II. METHODS.

The three main factors involved in investigations of this nature are light, temperature, and nutrition.

### A. *Light control.*

The arrangements for exposing the plants to a definite number of hours of light were quite simple. A wooden shed, made as free from light as possible but provided with ventilation, was built on to a glasshouse of similar dimensions, wooden doors dividing the two compartments. Some of the benches were on wheels so that plants could readily be run from one compartment to the other as desired.

In each experiment one half of the plants received all the daylight available, which naturally varied slightly in the different experiments,

according to the season of the year, while the other was exposed to a 9- (and in a single instance to a 7-) hour day only. A 9-hour day was chosen since it seemed likely to be short enough to appreciably retard if not actually prevent flowering, and yet the light curtailment was not too drastic to prevent quite vigorous vegetative growth.

The hours selected for the 9-hour day were from 8 a.m. to 5 p.m. Greenwich Time, which during the months when summer time was in force (i.e. the greater part of the experimental season) became 9 a.m. to 6 p.m. The light treatment was carried out from the time the seedlings were first put into the nutrient solution, i.e. approximately one week after the seed was sown.

### B. *Temperature conditions.*

No special apparatus was used to maintain equal temperature conditions in the shed and glasshouse during the period 5 p.m.–8 a.m. when the plants were separated, as the agreement between the two compartments in this respect seemed sufficiently close. The difference in the average minimum temperature in the two cases was surprisingly small, only ranging from 0.2–1.5° F. in a series of nine experiments (Table I). The agreement between the average maximum temperatures of the two compartments during these periods was not quite so close. As a general rule when the short day plants were first shut up for the night the temperature of the glasshouse was higher than that of the shed, but as the former cooled much the more rapidly large differences were of short duration only: For example, in the case of the experiment with *Vicia Faba* (July 17th–October 11th, 1930) closer examination of the charts shows that two hours after the plants had been separated the average difference in temperature between the two compartments was only 2.2° F., whereas the average difference in maximum temperature was as high as 10° F. An almost similar figure is obtained for the second experiment with *Phaseolus multiflorus* 1930.

### C. *Nutritive conditions.*

The Rothamsted nutrient solution <sup>1</sup> pH 6.2 was used throughout the season 1930, but a slight alteration in the proportion of the two phosphates was made in the following year to bring the pH to 5.0 and 5.5 in the case of the barley and soy bean cultures respectively, as it was thought that a more acid solution was desirable. The peas were grown in Zinzadze solution <sup>2</sup> during one experiment, and in Rothamsted solution at a pH of

<sup>1</sup> KNO<sub>3</sub> 1.0 gm.; KH<sub>2</sub>PO<sub>4</sub> 0.3 gm.; K<sub>2</sub>HPO<sub>4</sub> 0.27 gm.; NaCl 0.5 gm.; MgSO<sub>4</sub> . 7H<sub>2</sub>O 0.5 gm.; CaSO<sub>4</sub> . 2H<sub>2</sub>O 0.5 gm.; Fe<sub>2</sub>Cl<sub>6</sub> 0.04 gm. per litre H<sub>2</sub>O.

<sup>2</sup> NH<sub>4</sub>NO<sub>3</sub> 0.334 gm.; KNO<sub>3</sub> 0.166 gm.; Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> 0.70 gm.; KCl 0.614 gm.; MgSO<sub>4</sub> . 7H<sub>2</sub>O 0.5 gm.; CaSO<sub>4</sub> . 2H<sub>2</sub>O 0.5 gm.; Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> . 9H<sub>2</sub>O 0.25 gm. H<sub>2</sub>O per litre.

4.6 when the test was repeated. Salts declared free from boron by spectroscopic examination<sup>1</sup> were used in all forms of Rothamsted solution, but were not available for the Zinzadze cultures. The results, however, were identical irrespective of the type of solution used.

TABLE I.

*Average Temperatures (° F.) in Glasshouse and Shed during the Period 5 p.m.-8 a.m. (G.M.T.) when the Plants were in Separate Compartments.*

<i>Vicia Faba</i> , 1930.						
	March 31- June 23.		May 7- July 16. <sup>2</sup>		July 17- October 11. <sup>3</sup>	
	Max.	Min.	Max.	Min.	Max.	Min.
Glasshouse	65.7	47.7	73.5	52.9	72.8	51.9
Shed	60.1	46.8	68.6	53.1	62.8	50.4
Difference	5.6	0.9	4.9	0.2	10.0	1.5
<i>Phaseolus multiflorus</i> , 1930. <i>Hordeum vulgare</i> , 1930.						
	May 20- July 16. <sup>2</sup>		July 23- September 20. <sup>2</sup>		March 26- September 1. <sup>2</sup>	
	Max.	Min.	Max.	Min.	Max.	Min.
Glasshouse	75.1	53.4	74.7	53.4	70.5	50.4
Shed	70.5	54.0	64.7	51.9	63.0	49.4
Difference	4.6	0.6	10.0	1.5	7.5	1.0
<i>Hordeum vulgare</i> , 1931. <i>Glycine hispida</i> , 1931. <i>Pisum sativum</i> , 1932.						
	March 19- August 13.		May 13- August 14.		April 22- May 24.	
	Max.	Min.	Max.	Min.	Max.	Min.
Glasshouse	69.2	50.3	72.9	54.4	71.2	49.5
Shed	64.4	51.2	68.3	55.1	65.2	48.9
Difference	4.8	0.9	4.6	0.7	6.0	0.6

One half of the plants under each light treatment received one part per million boric acid ( $H_3BO_3$ ) in addition to the nutrient solution.

The seeds, graded by weight for each experiment, were germinated in damp sawdust, and the plants supported by wax corks were grown singly in glass bottles of 600 c.c. capacity. Renewal of the respective nutrient solutions was made at regular intervals, weekly changes being made after the first few weeks. Five plants were taken as the unit in every case.

Direct comparison was, therefore, available between plants subjected to summer daylight and those exposed to a 9-hour day (i.e. one somewhat

<sup>1</sup> The spectroscopic examination was carried out by Dr. Judd Lewis.

<sup>2</sup> Values for June 2-8, and August 24 missing.

<sup>3</sup> 5 p.m.-10 a.m. for this experiment.

shorter than that characteristic of spring or autumn) both sets being grown under approximately similar and definitely summer conditions of temperature. Since each light treatment was further carried out with and without the addition of boron to the nutrient solution, it was hoped to distinguish between the effect of changes in light, apart from that of temperature, on the response of the plant to boron and flower production.

### III. EXPERIMENTAL DATA.

Plants belonging to the long day category such as *Hordeum vulgare* (barley), *Vicia Faba* (broad bean), *Phaseolus multiflorus* (scarlet runner bean) and the Mandarin variety of *Glycine hispida* (soy bean) were principally grown, but experiments were also carried out with the Biloxi variety of *Glycine hispida*, a short day plant, and *Pisum sativum* (garden pea) which is intermediate in type.

#### A. *VICIA FABEA* (BROAD BEAN). *Sutton's Prolific Longpod*.

Experiments were carried out in 1930, March 31st–June 23rd, May 7th–July 16th, and July 17th–October 11th, the average normal day lengths in the three cases being 15 hrs. 8 mins., 16 hrs. 10 mins., and 13 hrs. 42 mins. respectively. A 9-hour day (8 a.m.–5 p.m. G.M.T.) served as the controlled short day in the first two cases, but a 7-hour day (10 a.m.–5 p.m.) was employed in the last experiment in order to obtain a greater difference between the normal and controlled conditions. The results of the three experiments were entirely consistent, so they will be considered together.

#### 1. *Development under short day conditions.*

(i) Exposure to a 9- or 7-hour day retarded, but did not prevent the appearance of characteristic boron deficiency symptoms in the plants grown in boron free solution, and also slowed down the rate of the subsequent degeneration (Pl. XVIII, figs. 1 and 2).

An exact quantitative estimate of this delay was not easily made, owing to the inevitable slight variation among the replicates and the difficulty of stating precisely when the deficiency symptoms first appeared, but on an average it was found to be 3 to 6 days. Although this sounds hardly significant, the injury once evident in the full day plants progressed very rapidly, so that after a 3- to 6-day interval when the symptoms only began to appear in the short day series, the distinction between the two sets of plants was really well marked. In no case did shortening the day produce signs of degeneration similar to those of boron deficiency in plants supplied with boron.

(ii) The 9- or 7-hour day inhibited the growth of the shoot in length, an effect which has already been described by Deats (4) and many other

workers. In the case of the broad bean, however, the difference between the heights of the long and short day plants was only temporary. At an early stage (after about 5 weeks), when the full day plants supplied with boron were growing vigorously, the short day series were only 58.6 per cent. of the height of the controls, but when flowering had begun in the latter, elongation slowed down and finally almost ceased, whereas the short day plants continued vegetative growth for a much longer period, and eventually the heights of the series levelled up (cf. Pl. XVIII, Fig. 1 taken during an experiment with Fig. 2 taken at the end of an experiment).

(iii) Shortening the day retarded and limited flowering and fruiting in the plants supplied with boron (Pl. XVIII, Fig. 2). Although a 9-hour day allowed of some flower formation it was considerably delayed (4 to 14 days in the three experiments) and *much* less prolific than in the plants with normal daylight. To get some numerical idea of this effect on the flowering, the clusters of flowers were counted in the two series (Table II), when the full day plants appeared to be at the height of bloom.

TABLE II.

<i>Vicia Faba.</i>	<i>Water Culture.</i>	<i>Full Nutrients + Boron.</i>
		Average No. of flower clusters per plant.
Full day (average 15 hours 8 minutes)	.	17.8
9-hour day	.	7.2

Excellent pods were produced by the plants exposed to full daylight in the mid-summer series, but no pods were formed on the corresponding short day plants up to the close of the experiment (Pl. XVIII, Fig. 2). This probably does not mean that no pods would have developed under a 9-hour day, but that their formation was merely delayed. It was, unfortunately, not possible to investigate this point further as the plants had to be harvested in order to give dry weight figures comparable with the already mature full day plants, and questions of space prevented the alternative course of duplicating this part of the experiment.

(iv) Shortening the day in some cases induced a temporary wilting condition of the plants grown with boron, although they were in other respects entirely healthy and vigorous. This phenomenon usually occurred in particularly hot weather, but recovery did not take place during the night, as is usual when the wilting is brought about by excessive transpiration in the presence of an inadequate water supply. No question of lack of water can arise in the case of plants grown in nutrient solution, and in fact plants which showed this tendency to wilt invariably failed to absorb even their normal quantity of solution. Aeration of the solutions was tried without any improvement being obtained. Garner, Bacon, and Allard (8) hold

that the duration of the daily illumination period profoundly affects the water relations of the plant, and give examples to show that maximum turgidity is favoured by a light period which is optimal for increase in size. Caldwell (3) has reported the occurrence of wilting in tomatoes grown under short length of day, the explanation of which appears to lie in the reduced carbohydrate content of the plant. In support of this the *Vicia Faba* plants in the short day series which showed the greatest tendency to become flaccid were those which produced least dry weight, and one case of wilting which even occurred under full light conditions also proved to be an abnormally low yielding plant. No instance of wilting was observed in any of the plants without boron, whether grown under full or short (9-hour) day. Apart from their small size and moribund state which would naturally be accompanied by a reduction in all vital processes, including transpiration, it is an interesting point that according to Johnson and Dore (10) plants deprived of boron show an accumulation of carbohydrate. If the wilting be due to a lack of this constituent, therefore, the plants without boron would be the less likely to exhibit the phenomenon.

## 2. *Dry weight and nitrogen content.*

The figures for the dry weight and nitrogen content of the plants are given in Table III, the main features of which are described below.

### (a) *Influence of the length of day on the effect of boron deficiency.*

Under both full and short day conditions removal of the boron brought about reductions in the yield of both shoot and root, but the decrease was much less marked in the case of the 9-hour day plants. Since the reductions in root-growth were in nearly every case greater than those in the shoot, plants grown in the absence of boron usually showed an increased shoot/root ratio.

Under both full and short day conditions, lack of boron caused a rise in the percentage of nitrogen present in the shoot. The actual nitrogen present, however, was in every case decreased, as would be expected from the very marked difference in size and vigour of the two sets of plants.

### (b) *Influence of boron deficiency on the effect produced by a shortened day.*

Shoot growth was considerably reduced by a shortened day if boron was present, but it was slightly increased where boron was not supplied. This increase was probably due partly to the somewhat greater growth that occurred before the onset of the dying under short day conditions, and possibly also to a slight loss of dry weight undergone by the full day plants in their more advanced stage of degeneration. The development of roots, on the other hand, was hardly affected or slightly reduced by shortening the day if boron was present. An increase, however, was obtained in

TABLE III.  
*Photoperiodism of Vicia Faba, Water Culture, 1930.*  
 Dry weights. Average of 5 plants.

Treatment.	March 31-June 23.				May 7-July 16.				July 17-October 11.			
	Shoot. gram.	Root. gram.	Total. gram.	Shoot. % N. in dry matter.	Shoot. gram.	Root. gram.	Total. gram.	Shoot. % N. in dry matter.	Shoot. gram.	Root. gram.	Total. gram.	Shoot. % N. in dry matter.
Full day + B	24.15	5.47	29.62	4.42	1.75	0.42			18.57	4.30	22.87	4.32
No B	4.53	0.63	5.16	7.19	2.62	0.12			3.64	0.55	4.19	6.62
Short day + B	11.61	4.21	15.82	2.76	2.89	0.34						1.92
No B	5.10	0.93	6.03	5.48	3.18	0.16			9.85	2.89	12.74	3.41
									4.29	0.83	5.12	5.17
	Full day. Average 15 hours 8 minutes				Full day. Average 16 hours 10 minutes				Full day. Average 13 hours 42 minutes			
	Short day. Average 9 hours				Short day. Average 9 hours				Short day. Average 7 hours			

plants not supplied with boron. The shoot/ratio was in consequence decreased by shortening the day, both where boron was supplied and where it was withheld, in the former instance due to a decrease in the shoot and in the latter owing to greater root development.

A rise in the percentage of nitrogen in the shoot occurred on shortening the day whether boron was supplied or not, but so long as boron was present the actual nitrogen was lower in the short day than in the full day plants. On the other hand, where boron was omitted, more actual nitrogen occurred in plants grown under the short day conditions than under normal daylight, a result in keeping with the slightly heavier dry weight produced by the former.

As regards dry weight, therefore, a lack of boron very materially lessened or even negated the effect of shortening the day to 9 or 7 hours, while a reduction in the length of day reduced the difference between the plants grown with and without boron.

B. *PHASEOLUS MULTIFLORUS* (SCARLET RUNNER BEAN (*Sutton's*  
*Prizewinner*).

Two experiments were carried out with *Phaseolus multiflorus* in 1930 during May 20th–July 16th and July 23rd–September 20th respectively, the average normal length of day being 16 hrs. 21 mins., and 14 hrs. 11 mins. in the two cases. A 9-hour day was taken as the short day throughout. The main results of the two experiments were quite consistent, and in general confirmed those obtained with *Vicia faba*.

1. *Development under short day conditions.*

(i) Exposure to a 9-hour day retarded but did not prevent the appearance of boron deficiency symptoms (Pl. XVIII, Fig. 3). The delay amounted to from 3 to 6 days, but as in the case of *V. faba* no exact measure was possible.

(ii) A 9-hour day had a marked inhibitory action on growth of the stem in length, and although a few of the set receiving boron made a start to elongate, every plant, whether supplied with boron or not, failed to 'run' in the normal manner. Since stunting of stem growth is also a characteristic feature of runner beans suffering from a deficiency of boron under normal light conditions, it was important to distinguish between the apparently similar effect of the two factors. This was in general possible, as plants failing to 'run' owing to a lack of boron died at the apex of the stem, whereas the apices of those stunted from reduced light conditions remained healthy. Some difficulty, however, arose from the fact that the plants without boron under a short day died so slowly that their apices were apt to retain a green and healthy appearance for an abnormally long time, although in other respects, such as failure to form flowers and general



habit, these plants showed the characteristic symptoms of boron deficiency. With *V. Faba* certain abnormalities appear in the internal structure of plants deprived of boron (19) and it seems not unlikely that similar irregularities might be expected to occur in *P. multiflorus*. Material of these doubtful cases has, therefore, been pickled in the hope that anatomical investigations may lend support to the conclusions drawn from the external appearance of the plants.

(iii) Shortening the day entirely prevented flowering, although the plants exposed to full daylight flowered freely. This applied to the plants receiving boron only, as flowering did not take place even in full daylight unless boron were supplied. These results are in agreement with those of Tincker (17) and Maximov (12) who obtained no flowering with *P. multiflorus* when exposed to a 10- or 9-hour day respectively from the earliest stages of growth. Garner and Allard (7), on the other hand, record the reverse effect, viz. flowering under short day conditions only.

## 2. Dry weight and nitrogen content.

The dry weight figures for these two experiments with *P. multiflorus* are given in Table IV. Too much weight, however, must not be put on dry weight figures alone, for in experiments of this nature the physiological behaviour of the plant is the fundamental point. The fact that one set of plants elongates or flowers normally whereas another set differently treated do not, is of real importance, but such a change in habit is not necessarily accompanied by an equally striking difference in dry weight.

### (a) Influence of the length of day on the effect of boron deficiency.

Under full day conditions removal of the boron brought about a reduction in dry weight of both root and shoot, and since the root was the more affected, a slight rise in shoot/root ratio was obtained. Under a shortened day, little or no reduction occurred in the weight of shoot when boron was lacking, though the root was definitely reduced in the second experiment. As a result an increase in the shoot/root ratio occurred in the latter case only.

The percentage of nitrogen in the shoot was slightly increased when boron was withheld under both full and short day conditions. In the case of the full day plants this was associated with a fall in the actual nitrogen present, but under short day conditions the actual nitrogen content remained unchanged whether or not boron was supplied.

### (b) Influence of boron deficiency on the effect produced by a shortened day.

Shoot yield was reduced by a shortened day if boron was present but was little affected in its absence. As regards root growth the results were

TABLE IV.  
*Photoperiodism of Phaseolus multiflorus. Water Culture, 1930.*  
 Dry weights. Average of 5 plants.

Treatment.	May 20–July 16.					July 23–September 20.				
	Shoot. gm.	Root. gm.	Total. gm.	Shoot. Root.	Shoot. % N. in dry matter.	Shoot. gm.	Root. gm.	Total. gm.	Shoot. Root.	Shoot. % N. in dry matter.
Full day { + B No B	5.35	0.98	6.33	5.46	1.84	4.83	1.01	5.84	4.79	2.23
	3.05	0.44	3.49	6.93	2.51	1.88	0.36	2.24	5.21	2.77
Short day { + B No B	2.60	0.44	3.04	5.91	2.80	2.29	1.19	3.48	1.93	2.46
	2.35	0.40	2.75	5.88	3.12	2.03	0.63	2.66	3.21	2.70
	Full day. Average 16 hours 21 minutes					Full day. Average 14 hours 11 minutes				
	Short day. Average 9 hours					Short day. Average 9 hours				

not altogether consistent, although the behaviour in each case found corroboration in one or other of the *V. faba* experiments. If boron were supplied a reduction in root weight occurred when the day was shortened in the first of the *Phaseolus* trials, a result which agrees with two of the three tests with *V. faba* (Table III), but this reduction was not confirmed when the experiment was repeated. In the latter case, however, an increase of root occurred on shortening the day in the absence of boron, as had been found in all the experiments with *V. faba*. The effect on the shoot/root ratio was in consequence various. No tendency to form tubers under short day conditions was observed as Garner and Allard (7) and Tincker (16) have described, but this is in all probability to be attributed to the fact that they grew their plants in soil, and the experiments now under consideration were carried out in water culture.

With regard to the nitrogen content, the percentage in the shoot was increased when the day was shortened whether or not boron were supplied in the first of the two experiments only, the value being unaffected by the length of day in the second trial. In both cases, however, a reduction in actual nitrogen occurred provided boron were present.

As in the case of *V. faba*, therefore, a reduction in the length of day very considerably lessened the difference in dry weight produced by plants grown with and without boron, although it did not affect the need of the plant for this element.

### C. *HORDEUM VULGARE* (BARLEY).

1930 Plumage Archer

1931 Goldthorpe, Standwell, Spratt Archer

{ Pedigree strains from National  
Institute Agricultural Botany,  
Cambridge.

The trials were carried out from March 26th to September 1st in the first, and from March 19th to August 13th in the second season, the average lengths of the normal day throughout the experiments in the two years being almost identical, viz. 15 hrs. 20 mins. As already stated, the nutrient solutions employed in the two seasons were not quite the same, as a slightly more acid (pH 5.0) modification of the Rothamsted (pH 6.2) solution was used in the second year.

#### 1. *Development under short day conditions.*

(i) The rate of growth was definitely retarded under the shortened day, but a much longer time elapsed before the influence of the length of day appeared compared with *V. faba*. This was no doubt due partly to the much longer growth period in the cereal plant, and also to the earlier time of year at which the barley was set up, the differences between the full and 9-hour day, at least during the first weeks of growth, being still

comparatively slight. With *V. faba* the superiority of the full over the 9-hour day plants was noticeable after about 10 days, whereas with all the four varieties of barley grown, 38 to 40 days elapsed before even slight differences in size could be detected.

(ii) The most outstanding effect of the short day on barley supplied with boron was the great delay in, or even prevention of ear formation. The precise behaviour depended on the variety, but in general the earlier the variety the less marked was the adverse effect of the short day. Among the four barleys tested, Standwell was the only one which produced a fair number of ears under a 9-hour day, although their appearance was delayed as much as 42 days, and the average number was only 14.6 per plant compared with 22.0 per plant developed under full daylight (Pl. XVIII, Fig. 4). Goldthorpe and Spratt Archer both formed ears eventually, but their emergence was also much retarded (58 and 51 days in the two cases) and their numbers reduced to an average of 3.4 and 12.0 per plant respectively, compared with 22.0 and 30.0 per plant under full day conditions (Pl. XVIII, Figs. 5 and 6). Plumage Archer, on the other hand, entirely failed to produce any ears at all (Pl. XVIII, Fig. 7).

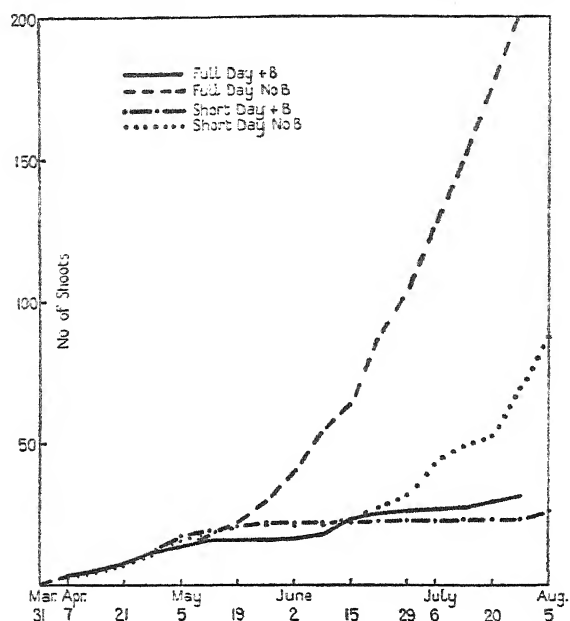
None of the short day plants were kept on to ripen, as the dry weight figures were wanted for comparison with the full day plants. It seemed improbable, however, that normal ripening off would have ever occurred. Very little grain showed signs of developing properly, and only in the case of Standwell was it possible to separate out any grain which might legitimately be termed fertile. Even then the yield was only 1.33 grm. per plant on an average of 5 plants, compared with 12.2 grm. under full daylight.

(iii) The shortened day also tended to bring about changes in habit, the effect being more marked in some varieties than others. Standwell and Goldthorpe, for example, assumed a spreading habit of a type which did not occur even in the early growth stages of the plants with full daylight. Plumage Archer, on the other hand, adopted a leafy, drooping habit, while Spratt Archer showed little departure from the normal beyond the extension of the vegetative period.

These results in respect of response to reduced length of day are in complete accordance with those of other workers, Tincker, for example (17), whose plants were grown under good cultural conditions with no nutrient deficiency. The point of interest at the moment, therefore, lies in the behaviour of plants deprived of boron, when subjected to a short day.

(iv) Shortening the day greatly retarded the appearance of the boron deficiency symptoms. Whereas the plants without boron exposed to full daylight started to fall behind the controls after an interval of one to two months, the 9-hour day plants without boron required approximately five to seven weeks *more* before they showed any inferiority to their corre-

sponding control set supplied with boron. The deficiency symptoms exhibited by these short day plants were precisely similar to those grown under full day conditions viz. a lack of ear formation<sup>1</sup> and a tendency to



continue vigorous tiller development throughout their life, as Sommer (15) has found to occur with other monocotyledons, though no premature tillering occurred as Morris (14) has described for wheat. None of the additional tillers in the barley were of any value, however, as they turned yellow at the apex and death of the shoot followed. Since one of the effects of shortening the day was to retard or even prevent ear formation, this abnormal tiller development afforded the best criterion as to whether or not the 9-hour day plants were suffering from a lack of boron. Shortening the day alone did not induce greater tillering (Table V), though Forster and others (6) found it caused an increase in the case of wheat, so it seemed evident that the abnormal rise in the number of tillers was rightly to be attributed to the deficiency of boron in the nutrient solution, both in the case of the full day and the 9-hour day plants.

## 2. Tiller formation in the presence and absence of boron under full and short day conditions.

It must be emphasized that much tiller formation late in the life of the plant is an *abnormal* feature, and as is seen in the text-figure above, which

<sup>1</sup> One instance occurred (Spratt Archer) where a single ear formed, but only sterile flowers developed.

illustrates tiller development throughout the life of these variously treated plants in the case of the Standwell variety, the control plants (full day with boron) had formed all tillers which were to be of any value for ear bearing during the first six weeks of growth, i.e. by May 12th. The slight increase noticeable from June 15th onwards, was due to the production of quite small shoots which did not develop ears. The plants without boron, on the other hand, showed no sign of slacking off in tillering, and the number of shoots increased without cessation until the end of the experiment. None of their tillers, however, whether formed early or late in the life of the plant produced ears.

The date at which a general distinction in size and habit first appeared between the plants grown with and without boron under full daylight coincided exactly with that when tiller development in the latter plants began to show signs of abnormality. This held for all varieties, although the dates were by no means similar in each case. It seemed justifiable, therefore, to take the point when the tiller curves first departed from the normal as an indication of the onset of boron deficiency. This proved of particular use in the case of the plants grown under short day conditions, where ear formation (the usual final criterion as to whether or not a plant was suffering from lack of boron) was much delayed or even suppressed. Further, by comparison of the tiller curves of the short and full day plants grown without boron, a fairly accurate measure of the delay in the appearance of boron deficiency symptoms brought about by the shortened day could be obtained. In the case of Standwell this delay amounted to five weeks.

### 3. *Dry weight and nitrogen content.*

In Table V the means of the average figures from the three varieties of barley grown in 1931 (each figure, therefore, representing the mean of 15 plants) are set out, those for Plumage Archer in the 1930 experiment being excluded as seasonal conditions were not conducive to altogether satisfactory growth, and the data, though entirely corroborative on all important points, were slightly less complete than those for the succeeding year's trial.

#### (a) *Influence of the length of day on the effect of boron deficiency.*

The total dry weight was slightly reduced by a lack of boron irrespective of the length of day, but the different parts of the plant were not all similarly affected. Under both full and short day conditions removal of the boron reduced the yield of ears and fertile grain to zero (0.07 grm. in column 2 represents 1 abortive ear which developed on a single Spratt Archer plant), but the yield of straw was only slightly reduced, a small

TABLE V.  
*Photoperiodism of Hordeum vulgare.*  
 Means of 3 Varieties; 5 Plants in each. Water Culture, 1931.

Treatment.	Per plant.			Dry weight.			Straws.		Grain.	
	Total no. Tillers.	No. Ears.	% Earing Tillers.	Grain. gm.	Straw <sup>1</sup> . gm.	Root. gm.	% N.	Actual N. gm. in dry matter.	% N.	Actual N. gm.
Full day { + B No B	36.2	23.00	63.50	10.25	30.98	3.99	0.82	0.237	2.29	0.257
	174.5	0.07	0.03	0.00	33.16	6.72	1.17	0.385	—	—
Short day { + B No B	36.9	10.00	31.20	0.44	33.75	6.46	1.40	0.463	3.13	0.037
	75.2	0.00	0.00	0.00	31.34	5.63	1.64	0.516	—	—

Full day. Average 15 hours 11 minutes  
 Short day. Average 9 hours

<sup>1</sup> Including sterile flowers.

increase even occurring in the plants exposed to full daylight owing to the large number of tillers produced.

An increase in root weight occurred in the absence of boron when full daylight was supplied, which considering the accompanying rise in tiller production suggests an association between root and tiller formation as occurs normally in the early development of the plant.

No increase in root, however, was obtained in these plants if they were grown under a 9-hour day. A reason for this may be suggested. Roots yielding approximately 6 grm. dry weight were apt to fill the bottles completely, so that the size of the culture vessels may possibly have been exerting a limiting action on growth.

Both the percentage and actual nitrogen in the shoot (which in this case consisted of straw only) were slightly increased in the absence of boron whether the length of day were full or short.

The effect of a lack of boron, therefore, was less marked under a 9-hour day than under full day conditions, but only in the case of the yield of root, was any definite alteration in the result obtained, and some explanation has been offered for this exceptional occurrence.

(b) *Influence of boron deficiency on the effect produced by a shortened day.*

The effect of the shortened day on the total yield was similar both in the presence and absence of boron, but owing to the drastic effect of a lack of boron in prohibiting ear formation distribution of the dry weight was necessarily different in the two cases.

Both a lack of boron and a shortened day reduced the development of fertile grain to zero, and no modification of this effect was obtained when both factors were in operation together.

A slight increase in yield of straw occurred with a 9-hour day in the presence of boron, whereas a slight decrease resulted if boron were not supplied. This decrease is probably without much significance since comparison is being made with a full day plant with quite abnormal tiller development and in consequence, an exceptionally heavy yield of straw.

As regards the root, where boron was supplied shortening the day resulted in a definite increase in yield, as Lubimenko and Szeglova (11) found to be the usual case with long day plants. In the absence of boron, however, a slight decrease in root growth occurred. Again, it is possible that the size of the bottle was exerting a limiting influence on root development, but the fact that the smaller yield of root was accompanied by a smaller number of tillers suggests that the result was a true one.

The percentage of nitrogen in the straw and grain was increased by growth under a short day in the presence (and in the case of the straw also in the absence), of boron. With the straw this was accompanied by an increase, but with the grain a decrease, in actual nitrogen present.



Although the removal of boron and shortening the day have in some respects similar results upon barley, yet the effects of the two factors can in other ways be readily distinguished; for example, both inhibited ear and grain formation, but *only* if boron was absent did abnormal tillering occur. This probably indicates that where no ears appeared in the plants supplied with boron grown under a short day it was a case of extreme delay rather than prevention. Real prevention occurred, however, apparently as the result of the death of the apical meristems, in the plants without boron, irrespective of the light conditions. No anatomical investigations were actually made in order to prove the point, but it is hoped to be able to follow up the question later. Since, in the event of ear formation being suppressed tillering received a stimulus, analogy with the result which frequently follows mechanical injury to the main growing apex was suggested.

Both removal of boron and shortening the day also tended to increase root growth, but the effect of each factor was counteracted by the presence of the other. e.g. shortening the day only increased root development provided no lack of boron occurred, and similarly a plant deprived of boron, which with a full day increased its root growth, failed to do so if subjected to a short day.<sup>1</sup> Further, although the same effect on root growth was obtained with the two factors, yet it seems probable that the nature of the influence exerted was not the same in the two cases, since where the day was shortened the subsequent increase of root was not accompanied by an increase in tiller formation, but where a lack of boron was associated with new root growth, fresh shoots were simultaneously developed.

#### *4. Response to boron.*

Before proceeding to describe the results obtained with other plants, some brief reference to the response to boron obtained with barley (and, as will be seen later, also with peas), in these experiments may not be out of place. In preliminary investigations already published (18) it seemed that boron was not essential for these species, though it was suggested at the time that the distinction between plants for which this element was (i) necessary, and (ii) advantageous was probably artificial, and that the difference in response was of degree only. Fortunately we have now been able to show that this is the case (Pl. XIX, Fig. 11), thus bringing these plants into line with the majority of other species tested, and the results into conformity with those of other workers.

This change in behaviour of plants grown in the later experiments is difficult to account for except by supposing that some source of boron

<sup>1</sup> A further possible explanation of this root behaviour is given under the discussion of dry weights.

unconsciously introduced in the earlier experiments (possibly by the nutrient solution or the glass culture bottles) had been removed. No alteration in this or in any other kind of technique, however, had been made as would afford any explanation on those lines, and salts, spectroscopically examined<sup>1</sup> for the presence of boron, with entirely negative results, and bottles lined with paraffin wax had in the case of barley been used before the response to a need for boron was obtained. Further, solution allowed to stand undisturbed in one of these uncoated bottles for six weeks<sup>2</sup> showed no trace of boron on spectroscopic examination, so that unless the presence of roots were essential before any boron could be dissolved out, it seems unlikely that the glass was a source of boron.

The danger of the glass furnishing sufficient of this element, however, seems to be small, since Sommer (15) states that even pyrex glass (a borosilicate) did not furnish her plants with sufficient of this element to mask the results, and in the experiments under discussion the glass bottles were definitely not of this type.

Whatever the correct explanation, it is evident that boron is essential for the normal development of barley as Sommer has shown, but at the same time its requirements must be considerably lower than those of other plants such as *Vicia Faba* or *Phaseolus multiflorus*, since the latter showed a marked response to a need for boron where no such need was exhibited by barley although grown under identical conditions.

All of the four varieties of barley just described failed to produce any fertile grain in the absence of boron, but occasional plants of another strain,<sup>3</sup> Archer Goldthorpe 4/5/1  $\times$  Goldthorpe-Spratt 18/1, developed a few malformed ears containing a little viable grain. A measure of the enormous difference between the yield of fertile grain from the plants grown with and without boron, however, may be gauged from the fact that in one season, only 3 seeds were obtained from 10 plants receiving no boron, whereas a similar number of plants supplied with boron yielded over 5,000.

This behaviour made it possible to grow successive generations of barley in water culture, so that the influence of the boron or no-boron treatment of the parent on the progeny could be investigated.

Plants derived from parents which had received no boron in the nutrient solution for four generations, apparently suffered no handicap from this pre-treatment, for they responded to the addition of boric acid and produced as good a crop of fertile grain as plants derived from parents supplied with boron throughout the same number of generations (Pl. XIX, Fig. 12).

<sup>1</sup> By which means less than one part  $H_3BO_3$  in 180 million parts nutrient solution could be detected.

<sup>2</sup> The longest period which any solution normally remains in a bottle unrenewed is four weeks.

<sup>3</sup> This barley was obtained from the cereal station, Ballincurra, but the locality does not appear to account for its difference in behaviour from the other varieties, since Cambridge grown strains have also produced grain in nutrient solution containing no boron in earlier experiments.

At the same time plants grown without boron and derived from parents which had received it for four generations, showed no superiority over those descended from parents grown for a similar number of generations without boron, thus indicating that no accumulation of this element had taken place, even sufficient for the completion of a single life cycle.

D. *GLYCINE HISPIDA* (SOY BEAN). Biloxi  
Mandarin } Seed from Washington.

The soy bean is a particularly good subject for experiments with length of day, as within the one genus both long and short day plants exist. The Biloxi variety was selected as an example of a short day plant, while Mandarin served as a long day type. The experiments were carried out from May 13th to August 14th, 1931, the average normal length of day during this period being 16 hours 1 minute, while the controlled short day was 9 hours throughout. Except for a modification of the culture solution to obtain a pH of 5.5 the methods employed were similar to those used in the case of the other plants already described.

### 1. *Development under short day conditions.*

#### (a) *Variety Biloxi.*

Since this variety normally flowers under a short day, the plants exposed to a 9-hour day formed the standard by which flowering behaviour was judged, whereas in the broad bean, scarlet runner, and barley the full day plants served as the controls.

(i) After a preliminary period of three weeks during which no differences were noticeable, the 9-hour day reduced the vegetative growth very markedly and the plants remained dwarfed throughout the course of the experiment. Those receiving the full day made excellent vegetative growth (Pl. XVIII, Fig. 8) provided boron was present.

(ii) Identical symptoms of boron deficiency appeared 3 weeks after the start of the experiment simultaneously under full and short day conditions (i.e. no retardation occurred as in the case of the broad bean), but their development was considerably delayed in plants receiving only a 9-hour day. The dwarfing effect of the shortened day was in this case readily distinguished from the stunting due to lack of boron, as in the former case only did the growing apices remain green and healthy.

(iii) Pods were developed freely from flowers of a cleistogamous nature (observed also by Garner and Allard (7)) under a 9-hour day, provided boron was supplied, but no flowering of any kind occurred in the plants exposed to full daylight up to the end of the experiment (August 14), the natural day being evidently still too long to allow of it.<sup>1</sup>

<sup>1</sup> Similar plants carried on longer showed flower buds on October 31, when the length of day was 9 hrs. 41 mins., but they failed to open. The low temperature was probably responsible for this.

(b) *Variety Mandarin.*

Growth of the Mandarin variety was somewhat slower than that of the Biloxi series, and differences between the plants grown with and without boron did not appear until 2 weeks after they were evident in the latter. The results with Mandarin were in some respects less well defined than in the case of Biloxi, as the reduction in length of day to 9 hours exerted such a drastic effect that differences between the plants grown with and without boron were not easy to detect. The results obtained, however, were in no way contradictory, but rather lacked definition.

(i) After about 4 weeks from the start the short day plants fell behind those receiving full daylight and eventually the difference in size between the two sets became very marked (Pl. XIX, Fig. 9).

(ii) Exposure to a 9-hour day greatly retarded the appearance of the boron deficiency symptoms, and even to the end of the experiment they were not very definite. This was, no doubt, due to the overwhelming effect of the short day which prevented sufficient growth being made as should use up the original supply of boron present in the seed. From the appearance of the plants, however (Pl. XIX, Fig. 9), it is evident that they were distinctly poorer than those receiving boron, and although a few pods were actually formed on some of these plants, they showed a tendency to drop off without developing properly, so that it seems justifiable to conclude that the absence of the element was exerting some harmful effect, even if the deficiency symptoms were somewhat ill defined.

(iii) Flowering and pod formation was delayed and much reduced, but not altogether prevented, by growth under a 9-hour day. The extent of the delay could not be determined as flowering was of a cleistogamous nature under the short day, and although small petals were produced no opening of the buds was ever observed.

2. *Dry weight and nitrogen content.*

The figures for the dry weights and nitrogen content of the shoots of both Biloxi and Mandarin soy beans are given in Table VI.

(a) *Influence of the length of day on the effect of boron deficiency.*

In the case of both varieties the yields of shoot and root were definitely reduced by a lack of boron under full and short day conditions, though less markedly so in the case of the short day. The reduction in the yield of root was greater than that of the shoot under a full day, so that a rise in shoot/root ratio was obtained. This, however, did not occur under a 9-hour day, since the shoot and root were affected very similarly by a lack of boron.

The percentage of nitrogen in the shoot showed a marked rise in the

TABLE VI.  
*Photoperiodism of Glycine hispida. Water Culture, 1931.*

Dry weights. Average of 5 plants.

Treatment.	Biloxi (short day type).				Mandarin (long day type).				
	Shoot. gram.	Root. gram.	Total. gram.	Shoot. Root.	Shoot.		Shoot. Root.	Total. gram.	
					% N. in dry matter.	Actual N. gram.			
Full day { + B <sup>1</sup> No B	16.28	7.33	23.61	2.22	1.48	0.241	18.20	7.94	26.14
	2.51	0.47	2.98	5.34	3.86	0.097	1.47	0.49	1.96
Short day { + B No B	5.85	1.93	7.78	3.03	2.53	0.148	2.50	1.15	3.65
	1.89	0.57	2.46	3.32	4.23	0.080	1.53	0.89	2.43

Full day. Average 16 hours 1 minute  
 Short day. Average 9 hours

<sup>1</sup> Average four plants only.

absence of boron irrespective of the length of day. The actual nitrogen, however, was considerably reduced in the shoots of all plants where boron was lacking.

(b) *Influence of boron deficiency on the effect produced by a shortened day.*

Shortening the day reduced the yield of shoot and root very considerably in both varieties, provided boron was present. In the absence of boron, on the other hand, the dry weight of shoot was only slightly if at all reduced by the 9-hour day, and the yield of root was increased, especially in the case of the long day variety Mandarin. The shoot/root ratio was in consequence slightly increased or unaffected by short day conditions if boron were supplied, but was definitely reduced in its absence.

A rise in percentage of nitrogen and fall in the actual nitrogen occurred in the shoot under a 9-hour day in both varieties where boron were supplied, but no significant change occurred if boron was not present.

E. *PISUM SATIVUM* (GARDEN PEA). *Sutton's Harbinger.*

Peas are an example of plants where the lowering is little affected by a fairly wide range of length of day, and they thus afford a convenient link between definitely long and short day plants. The pea experiments were carried out during April 27th–June 27th, 1931, and repeated during April 22nd–June 24th, 1932, the average normal lengths of day being 15 hours 53 minutes and 15 hours 28 minutes respectively, whereas the controlled short day was 9 hours throughout in each case.

1. *Development under short day conditions.*

(i) Although two to three weeks elapsed before any differences became noticeable, growth was retarded under a 9-hour day, the short day plants remaining smaller than the controls throughout the experiment (Pl. XIX, Fig. 10).

(ii) No prevention or even delay in flowering occurred, but the progress of maturation, as seen in pod formation and development, was definitely retarded.

(iii) As regards the time of appearance of boron deficiency symptoms<sup>1</sup> in the two sets of plants the results from the two experiments were not quite consistent. A slight, but quite definite delay (six days) occurred in the appearance of the deficiency symptoms in the short day plants in the first experiment, whereas no such retardation was found in the succeeding year. On account of the difficulty in detecting the first signs of a lack of boron in peas and the inevitable individual variation

<sup>1</sup> See section 4. Response to boron, under Barley.

among the plants, too much weight must not be attached to this discrepancy. The delay, when it did occur, was less marked than in the broad bean, though the time interval in the latter case was even smaller.

2. *Dry weight and nitrogen content.*

As the dry weight figures for the two experiments are very similar, those for the second trial only will be given (Table VII).

TABLE VII.

*Photoperiodism in Pisum sativum. Water Culture. 1932.*

Dry weights. Average of five plants.

Treatment.	Shoot.		Root. gm.	Total. gm.	Shoot. Root.	Shoot.	
	Stems and Leaves gm.	Pods. gm.				% N. in dry matter.	Actual N. gm.
Full day { + B <sup>1</sup> . { No B	1.76	3.12	0.54	5.42	9.67	2.34	0.13
	1.16	0.00	0.20	1.36	6.13	2.97	0.04
Short day { + B <sup>1</sup> . { No B	1.03	1.30	0.36	2.69	6.53	3.39	0.09
	1.17	0.00	0.25	1.42	5.10	3.37	0.05
Full day. Average 15 hours 28 minutes							
Short day. Average 9 hours							

(a) *Influence of the length of day on the effect of boron deficiency.*

Under both full and short day conditions a lack of boron reduced the yield of pods to zero, but the dry weight of the leaf and stem part of the shoot were little if at all affected. The weight of roots was also lowered where boron was not supplied, the reduction being specially marked under full day conditions, and since the yield of the shoot was reduced more than that of the root, a decrease in shoot/root ratio occurred in each case. The percentage of nitrogen in the shoot was hardly affected by a lack of boron irrespective of the length of day, but the actual nitrogen present was much reduced in both cases.

(b) *Influence of boron deficiency on the effect produced by a shortened day.*

In the presence of boron shortening the day brought about a small though definite reduction in the yield of all parts of the plant, more especially in the shoot, so that the shoot/root ratio was decreased. No reduction in yield, however, occurred where boron was not supplied.

<sup>1</sup> Average four plants only.

The percentage of nitrogen in the shoot was increased by a shortened day if boron were present, but only slightly raised in its absence. The actual nitrogen, on the other hand, was little, if at all, affected by a reduction in the length of day, whether or not boron was supplied in the nutrient solution.

A shortened day, therefore, has a much less striking effect upon the growth of the pea plant than the omission of boron from the nutrient solution.

#### IV. GENERAL DISCUSSION.

As stated in the introductory portion of this paper, the object of these experiments was to obtain a comparison between various plants grown under full (summer) and short (spring and autumn) lengths of day respectively, both sets, however, receiving similar and definitely summer conditions of temperature. Since one half of the plants were supplied with boron, whereas the remainder received none, it was possible to determine the relative importance of the two factors, light and temperature, in causing the retardation both in the appearance of the boron deficiency symptoms and in the production of flowers in spring or autumn grown plants, compared with those grown in the summer. At the same time it was hoped to obtain evidence as to whether or not any correlation existed between these two phenomena.

In the case of *Vicia Faba* (broad bean), *Phaseolus multiflorus* (scarlet runner bean), *Glycine hispida* variety Mandarin (soy bean) and *Hordeum* (barley), shortening the day to 9 hours definitely retarded the appearance and progress of the symptoms of a deficiency of boron, thus proving that the reduction in the length of day rather than the temperature was the controlling factor in this case. Only with the Biloxi variety of soy bean and *Pisum sativum* (garden pea) was no delay in the appearance of deficiency symptoms obtained, but as these were examples of plants which were not definitely long day in type, the difference in their behaviour under the altered light conditions is probably accounted for.

In order to determine whether or not the delay in flowering which accompanies the delay in the appearance of the boron deficiency symptoms indicates an association between the function of boron and the production of flowers, it is best to take the case of such plants as the scarlet runner or Biloxi soy bean, where the shortened day not only retarded but actually prevented flowering throughout the course of the experiment. It will be remembered that in these plants there was a sharp distinction between the growth, habit, and development of the plants grown with or without boron, although neither of them flowered. This indicates that apart from flowering, boron exercises an important influence on the growth of the plant, though there is still nothing to preclude its being of special importance for



flower formation. All the evidence so far obtained goes to show the importance of boron for meristematic activity. In its absence the stem and root apices are the first parts of the plant to be affected, and since flower initials are essentially meristematic in nature, there seems every reason to believe that boron is as necessary for their normal development as for that of vegetative apices. That shortening the day does not of itself injure the flower or vegetative meristems is shown by the fact that plants failing to flower or elongate when exposed to an unfavourable length of day, do so as soon as the light conditions become favourable. A plant deprived of boron, on the other hand, almost always fails to flower, vegetative apices die irrespective of the light conditions, and even if the deficiency of boron is remedied before death of the plant is complete, recovery invariably takes place by means of entirely fresh lateral growth and not by renewed growth of the affected parts. It would seem, therefore, that no *special* correlation exists between the function of boron and flower formation except in so far as this element is associated with the growth of all types of meristematic tissues, flower primordia included.

#### V. SUMMARY.

1. The reduced length of day rather than the lower temperature is the factor controlling the delay in appearance of boron deficiency symptoms during the spring and autumn, compared with the summer months.

2. No special association between the function of boron and flower production was found, except in so far as flower formation is meristematic in nature, and is in consequence affected by a lack of this element.

3. Within the range of 7-16 hours, the length of day has no bearing on the need of the plant for boron, since with one possible exception, where the case remained unproven (Mandarin soy bean), death of all the plants ensued where this element was not supplied irrespective of the length of day to which they were exposed.

4. The deficiency symptoms characteristic of a lack of boron were similar under both long and short day conditions, although they were less pronounced and their rate of progress retarded if the day were short.

5. In no case did shortening the day produce degeneration effects similar to those induced by a lack of boron. Although the influence of the two factors in certain instances appeared similar, as where flowering was completely prevented, the resemblance was superficial only.

6. The presence of each factor modified the influence of the other:

(a) In the absence of boron the influence of the length of day was less striking than where boron was present.

- (b) The boron deficiency symptoms were less pronounced under short day than under full day conditions.

7. The lack of boron exerted a more fundamental influence on the plants than a reduction in length of day to 9 or 7 hours.

In conclusion, thanks are due to Dr. W. E. Brenchley for her unfailing interest and helpful advice throughout this investigation.

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## EXPLANATION OF PLATES XVIII AND XIX.

Illustrating Miss Katherine Warington's paper on 'The Influence of Length of Day on the Response of Plants to Boron'.

### PLATE XVIII.

Figs. 1-10. Plants grown in water culture showing development under full and nine hour length day, with and without the addition of 1 p.p.m. boric acid in the nutrient medium. 1. *Vicia Faba* (broad bean) mid-growth stage. 2. *V. Faba* final growth stage. 3. *Phaseolus multiflorus* (runner bean). 4. *Hordeum vulgare* (barley) variety Standwell. 5. *H. vulgare* variety Goldthorpe. 6. *H. vulgare* variety Spratt Archer. 7. *H. vulgare* variety Plumage Archer. 8. *Glycine hispida* (soy bean) variety Biloxi.

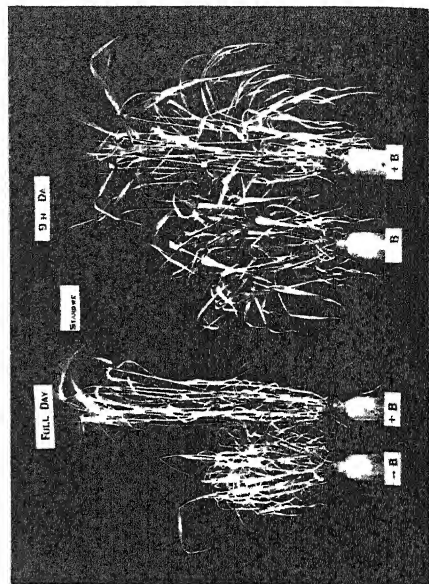
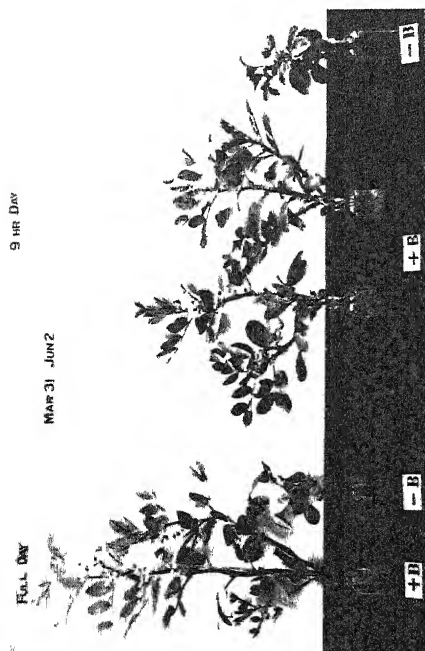
### PLATE XIX.

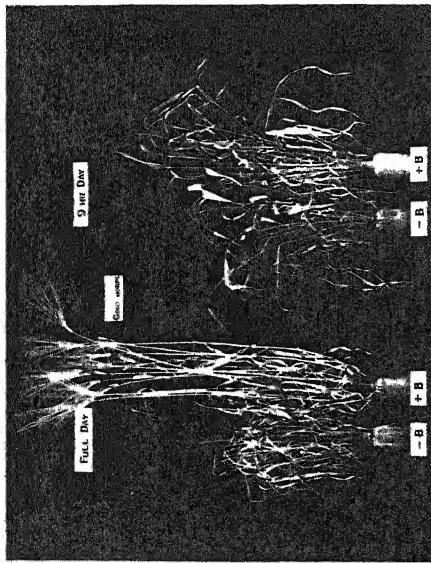
Fig. 9. *G. hispida* variety Mandarin.

Fig. 10. *Pisum sativum* (pea).

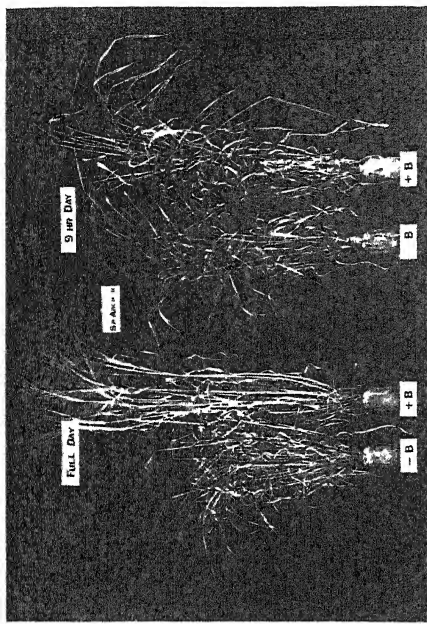
Fig. 11. Three different varieties of barley grown in water culture under normal light conditions with and without boron.

Fig. 12. Growth of the fifth generation of barley with and without 1 p.p.m. boric acid in the nutrient solution. Left to right: (1) 4 years no boron, 5th year with boron, (2) 4 years no boron, 5th year no boron, (3) 4 years with boron, 5th year no boron, (4) 4 years with boron, 5th year with boron.

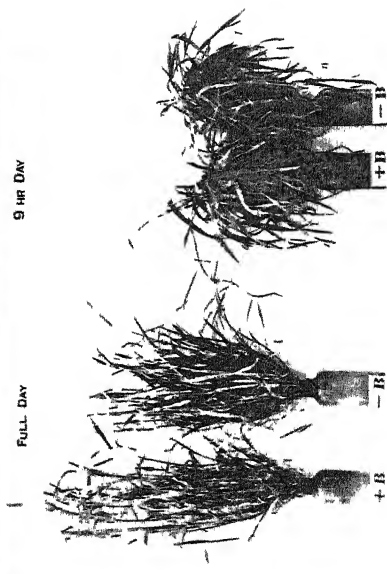




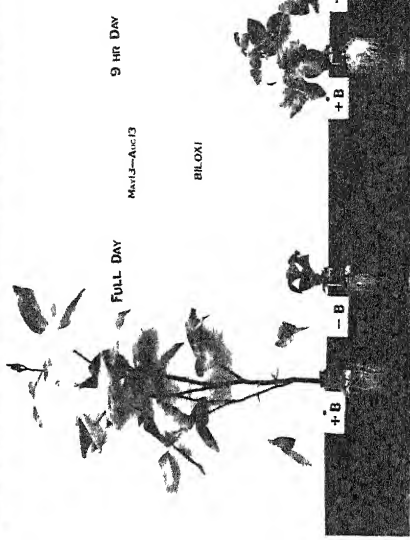
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# WARINGTON --- RESPONSE OF PLANTS TO BORON.





9 HR DAY

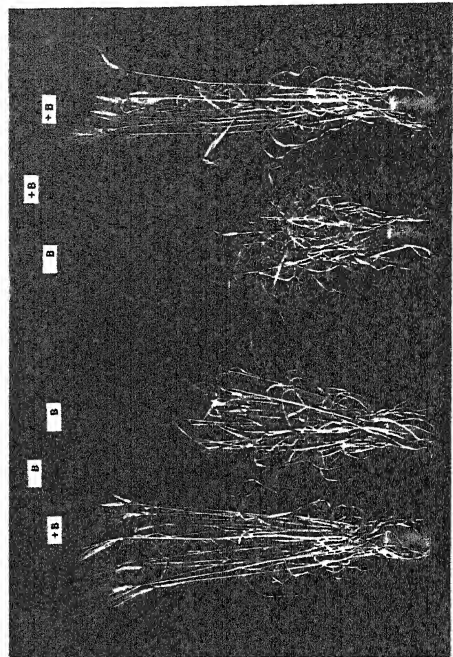
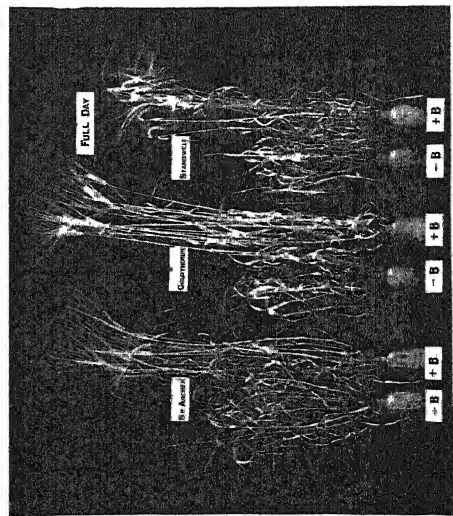
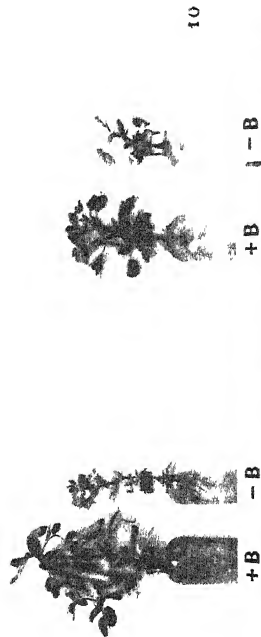
MEXIS-ALCIS

MANDARIN

FULL DAY

9 HR DAY

AP 22 - JUN 23







# A Fifth Contribution to our Knowledge of the Anatomy of the Cone and Fertile Stem of *Equisetum*.

BY

ISABEL M. P. BROWNE.

With eight Figures in the Text.

## INTRODUCTION.

THE present paper embodies the results of a study of the distribution of the protoxylem in the cones of the genus *Equisetum*.

Miss Barratt appears to have been the first person to give attention to the subject. Her paper, published in 1920, contains six figures, illustrating the distribution of the protoxylem in the axis of the cones of *Equisetum*.<sup>1</sup> Of these, three represent respectively such portions of the vascular systems of a cone of *Equisetum arvense* and of two cones of *E. palustre*, as can be seen when a cone is cut longitudinally in two, but not otherwise sectioned, and half of it is dehydrated, stained, cleared, and mounted.<sup>2</sup> The other figures show smaller portions of steles of cones of *E. arvense*, *E. maximum*, *E. limosum*.

Rather more than a year later I published reconstructions of the axial metaxylem and protoxylem systems of complete cones of *E. debile* and *E. silvaticum*.<sup>3</sup> Similar reconstructions, shown in Figs. 1-7, have now been made of the axial metaxylem and protoxylem systems of complete cones of *E. arvense* L., *E. maximum* Lam., *E. palustre* L., *E. hyemale* L., *E. limosum* L., and *E. variegatum* Schleich.

## II. DESCRIPTION.

In discussing the anatomy of the cones of *Equisetum* it is convenient to be able to distinguish the levels of the stele from which the traces depart from the tracts of vascular tissue lying between them. I shall, therefore, allude to the former as pseudo-nodes, and to the latter as pseudo-internodes,

<sup>1</sup> (1), Text-figs. 18, 22, 22 a, and 23, pp. 222, 285-6; Pl. VII, Figs. 5 and 6.

<sup>2</sup> For details of this method see (1), p. 202.

<sup>3</sup> (5), Text-figs. 2 and 3, pp. 431, 433.

thereby recognizing a certain analogy between the axis of the cone with its whorls of sporangiophores on the one hand, and the vegetative axis with its whorls of leaves on the other. No homology is, however, implied, since this depends on whether leaves and sporangiophores are truly homologous or not.

The table on page 461 contains a summary of the anastomoses of the meta- and protoxylem systems of the cones in the nine species of which a reconstruction of both systems has been made of a whole cone. In this table and in the following discussion the extreme apex of the cone, with its permanently incompletely differentiated sporangiophores, has been left out of account, as have also the annular and supra-annular anastomoses, since these are correlated to the insertion of the annulus, a reduced whorl of leaves.<sup>1</sup> It should be borne in mind that there is considerable variation in the structure of the stele of the cone within the limits of the species, and that for each of these studied only one such reconstruction has been made.

This table and the various reconstructions show that in cones of *Equisetum*, the protoxylem always anastomoses much less freely than the metaxylem; and that, as first pointed out by Barratt, the branching of the one frequently bears no relation to that of the other.<sup>2</sup> Moreover, it is not possible to arrange the nine species studied in a series showing progressive increase or decrease of anastomosis in both the protoxylem and the metaxylem systems of the cone. In cases in which anastomoses of the one system are numerous, relatively to the size of the cone, there may be comparatively few anastomoses of the other system. Thus, the cones of *E. arvense* and *E. silvaticum* that were studied (Fig. 1 of the present paper and (5), Text-fig. 2, p. 431) have very nearly the same number of sporangiophores, but in the former the anastomoses of the metaxylem are thrice as numerous as, and those of the protoxylem less by one-third than the corresponding anastomoses in *E. silvaticum*. If we attempt to arrange the cones studied in a series showing relatively to their sizes (i.e. to the number of their sporangiophores) decreasing frequency of anastomosis, we get, using the metaxylem system, the following order: *E. arvense*, *E. limosum*, *E. giganteum*, *E. palustre*, *E. variegatum*, *E. silvaticum*, *E. hyemale*, *E. maximum*, and *E. debile*. If we use the protoxylem-system, the order is: *E. silvaticum*, *E. limosum*, *E. debile*, *E. hyemale*, *E. palustre*, *E. arvense*, *E. giganteum*, *E. variegatum*, and *E. maximum*.

A study of the table on page 461 and of the various reconstructions shows that though genuine branchings of the axial strands of protoxylem are rare, cases of their fusion are rarer still. By genuine branchings I understand cases in which the protoxylem-strand divides into two branches, neither of which passes out in its entirety, a little higher up, into a sporangiophore. Such true branches must not be confused with the

<sup>1</sup> (10), p. 1109, (4), pp. 256-60.

<sup>2</sup> (1), p. 226.

*Table of the Anastomoses in the Stoles of the Cones of Equisetum.*

Species.	Number of sporangiophores in cone.	Metaxylem.			Protoxylem.		
		Branch-ings.	Fusions.	Total anastomoses.	Branch-ings.	Fusions.	Total anastomoses.
<i>E. maximum</i> Lam. . .	296	58	85	143	17	1	18
<i>E. arvense</i> L. . . .	118	92	97	189	8	4	12
<i>E. silvaticum</i> L. . .	116	28	35	63	11	7	18
<i>E. limosum</i> L. . . .	107	34	41	75	8	7	15
<i>E. giganteum</i> L. . .	91	26	31	57	9	0	9
<i>E. pulustre</i> L. . . .	87	24	28	52	8	1	9
<i>E. hyemale</i> L. . . .	84	22	22	44	9	0	9
<i>E. debile</i> Roxb. . . .	84	12	6	18	9	0	9
<i>E. variegatum</i> Schleich.	25	6	8	14	2	0	2

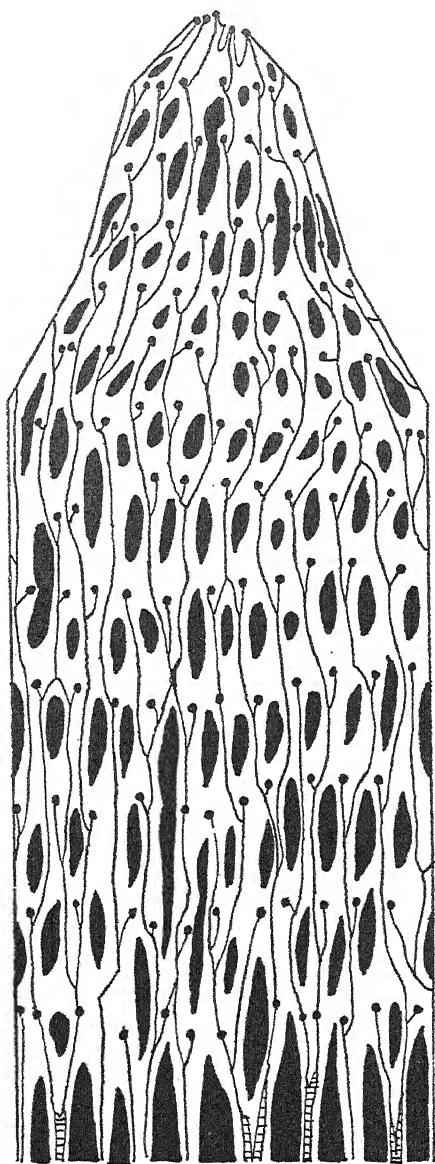


FIG. 1. Reconstruction of the stele of a cone of *E. arvense* L.  $\times$  circa 16. Parenchyma black; metaxylem white; protoxylem a line, its width barred.

numerous short branchlets that pass out, a little higher up, into a sporangiophore. These represent merely an early individualization of the xylem of the trace.<sup>1</sup> But though fusions are rarer than branchings, the number of strands of protoxylem does not tend to increase as we pass upwards in the cone. Apart from their relatively rapid reduction in the apical region, they seem frequently to be diminished in number, both actually and relatively to the sporangiophores, as we pass upwards in the cone, especially in *E. maximum*, *E. palustre*, and *E. hyemale* (Figs. 2, 2 a, 3, and 4). Throughout the genus the reduction in number of the protoxylem strands of the cone is chiefly effected by their passing out in their entirety into sporangiophores. This factor is more than sufficient to compensate for the excess of branchings over fusions, and usually brings about a reduction in number of the strands of protoxylem before the apex of the cone is reached. Except in *E. silvaticum* and *E. limosum*, fusion is an insignificant factor in this reduction and even in them it is not the principal factor. Thus, in a large cone of *E. maximum* (Figs. 2 and 2 a), there was but one, and in the cones of *E. hyemale*, *E. debile*, and *E. variegatum* studied, there were no such fusions (Figs. 4 and 7; (5), Text-fig. 3, p. 433).

<sup>1</sup> It is sometimes difficult to distinguish in the diagrams between a rather long branchlet of protoxylem, destined to a trace and having been individualized relatively early, and a branch of the main axial protoxylem passing out at the next pseudo-node, especially in *E. maximum*, where there is a great deal of irregularity in the departure of the traces of the sporangiophores.

It is curious that there should be so very few anastomoses of the protoxylem in the stele of the cone of *E. arvense*, of which Fig. 1 represents a reconstruction, for here the strands of metaxylem anastomose very

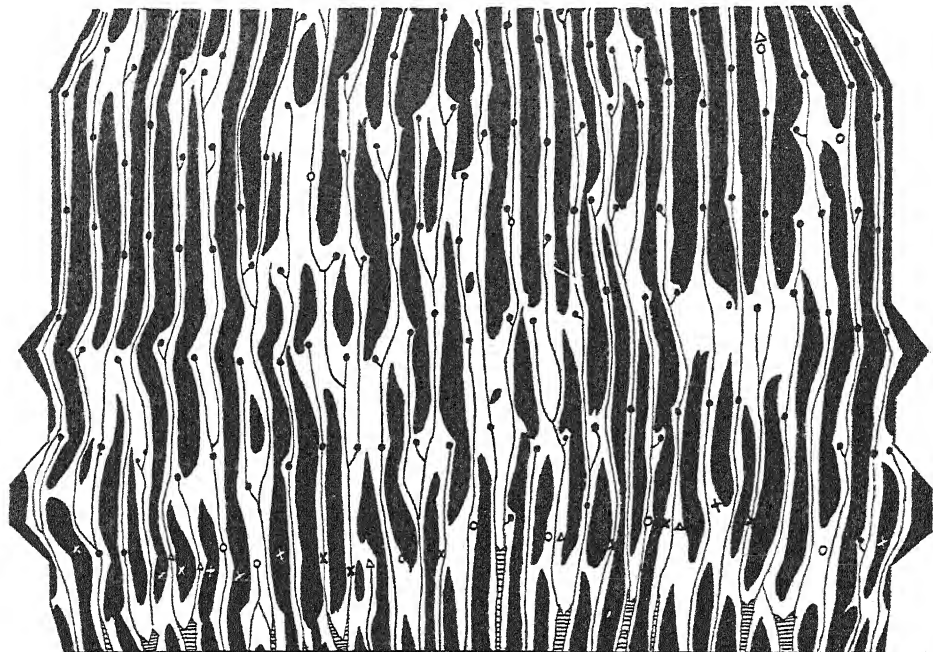


FIG. 2. Reconstruction of the lower part of the stele of a cone of *E. maximum* Lam.  $\times$  circa 10. Explanations as for Fig. 1. Traces dying out in the cortex are marked by a cross, black on white and white on black; those of which the phloem only joins on to the axial tissues are marked by a  $\Delta$ ; and those of which the protoxylem penetrates into the stele, but dying out in the metaxylem, fails to unite with the axial protoxylem are shown thus O.

freely. Where branching of the protoxylem occurs it is, naturally, generally associated with a branching of the metaxylem, in other words, of the bundles; though exceptions to this generalization are found occasionally, e.g. in *E. giganteum* and *E. maximum*, where wide bands of metaxylem, corresponding to two (or more) bundles, and containing more than one strand of protoxylem, have been recorded; and, more rarely, in relatively narrow bundles. A regular alternation of the protoxylem-strands of the cone by forking and fusion has nowhere been observed, even over a small vertical extent of the stele. It is only in the stele of the cone of *E. limosum*, towards the middle of the diagram and between the fifth and sixth whorls, that we find an approximation, imperfect, and of small extent, to such an alternation (Fig. 5).

Since the strands of protoxylem are never regularly anastomotic over any considerable extent of the stele, and as even irregular forkings are

uncommon, other modes of adjustment for supplying traces to sporangiophores that alternate in successive whorls are often necessary. In *E. arvense* (Fig. 1), where most of the bundles anastomose at the levels of the

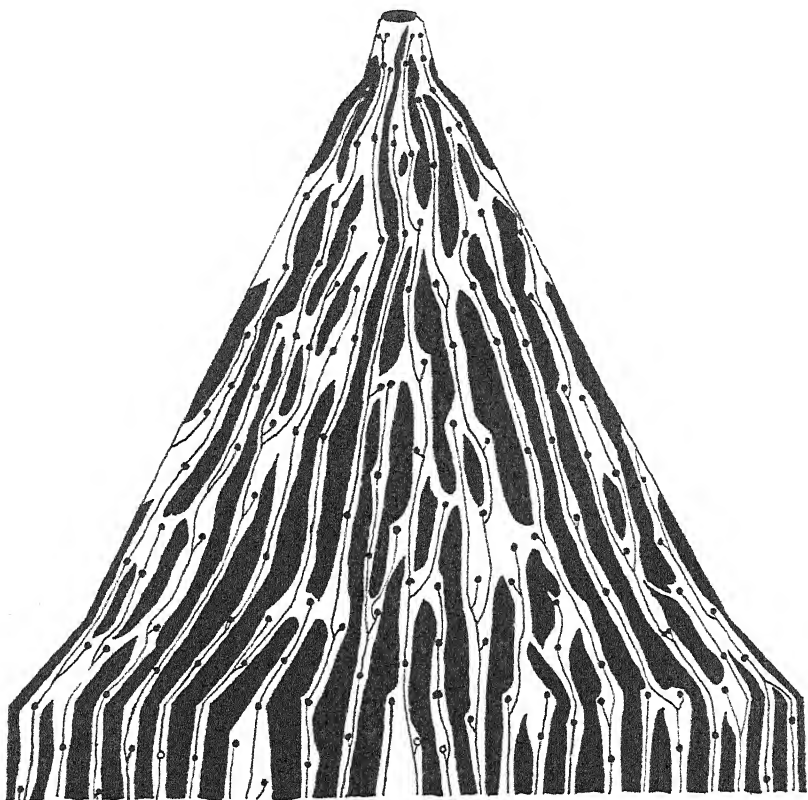


FIG. 2 *a*. Reconstruction of the upper part of the stele of *E. maximum* Lam., of which Fig. 2 represents the lower part.  $\times$  circa 10. Explanations as in Fig. 2.

pseudo-nodes, many of the strands of protoxylem profit by the fusion of the strands to swerve into the reconstituted bundles of the next pseudo-internode, and are thus brought more or less on to the radii of the sporangiophores of the next whorl (cf. Fig. 1). Frequently, in *E. arvense*, little or no further adjustment is required; but, even in this species, certain of the strands of protoxylem are not opposite to sporangiophores, either on account of the persistence of a wide tract of parenchyma through a pseudo-node, or because the swerving of the axial strand of protoxylem was insufficient, or because of some other irregularity. In such cases the protoxylem destined to the sporangiophore pursues an oblique course. Except in *E. arvense*, it is much more usual for the protoxylem destined to the appendage to pursue a markedly oblique course than for the main

axial strand to swerve, at the pseudo-nodes, into a bundle more or less alternating with that in which it was situated in the pseudo-internode below. In regions of the cone, or in species in which many of the bundles

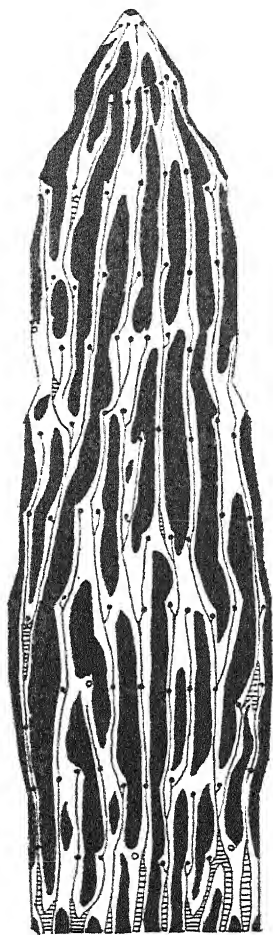


FIG. 3.

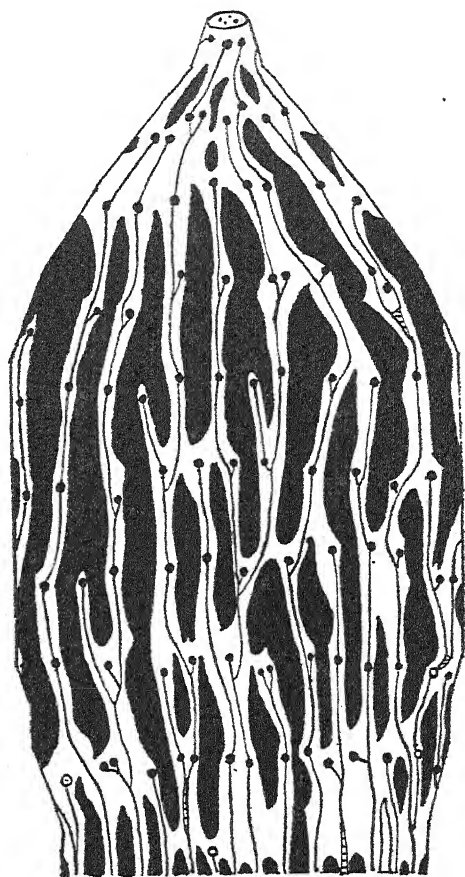


FIG. 4.

FIGS. 3 and 4. Reconstructions of the stele of *E. palustre*  $\times$  circa 10, and of *E. hyemale*  $\times$  16. Explanations as in previous Figures.

are narrow, and pass unbranched through more than one pseudo-node (e.g. cones of *E. maximum*, cf. Figs. 2 and 2 a), so that the points of departure of the traces are necessarily superposed, or nearly so, in successive whorls, the whole, or nearly the whole of the divergence necessary to bring the departing strand opposite to the sporangiophore that it enters is effected by the oblique course of the trace through the cortex. But, when the width of the bundle allows of it, the adjustment in position of the departing protoxylem to the sporangiophore usually begins within the stele. If the

bundle be wide, or the strand of protoxylem not far removed from the radius of the sporangiophore, little or no extra-stelar divergence may be required. Even in cones with a highly irregular network of narrow strands

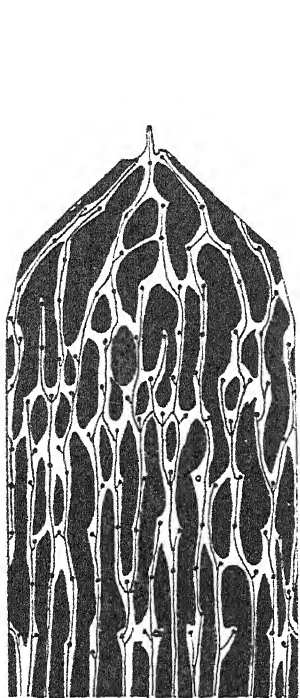


FIG. 5.

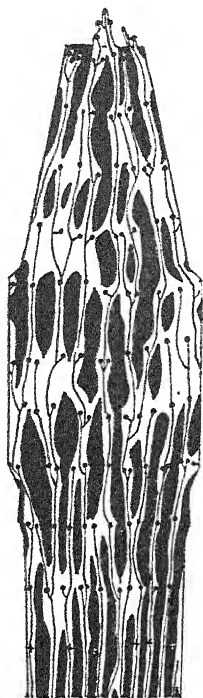


FIG. 6.

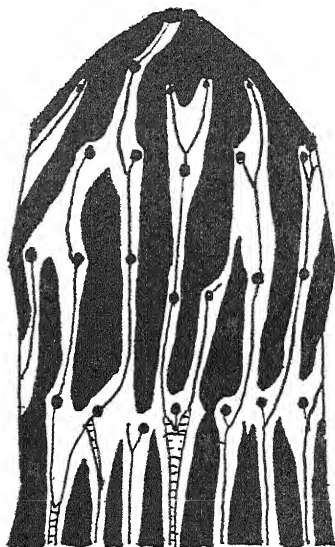


FIG. 7.

FIGS. 5, 6, and 7. Reconstructions of the steles of cones of *E. limosum*  $\times$  circa 8; of *E. giganteum*  $\times$  circa  $7\frac{1}{3}$ ; and of *E. variegatum*  $\times$  circa  $26\frac{2}{3}$ . Explanations as in other Figures.

there are often cases in which the axial strands of protoxylem find themselves, from a combination of factors, opposite to the sporangiophores to which they give off branchlets of protoxylem. In these cases, and in those in which the whole of the adjustment is effected by the divergence of the traces in the cortex, the traces appear in the diagrams to be sessile on the axial strands of protoxylem, because the departing tracheides, being on the same radius as those of the parent strand, cannot be shown in the diagram. Generally, however, these departing strands become individualized and separated from their parent strands at levels somewhat below the points of exit of the trace from the stele. In the other cases, where some or all of the adjustment of the departing tracheides to the position of the sporangiophores is effected within the stele, the traces appear in the diagrams to terminate lateral branchlets of protoxylem of varying length, and forming various angles with the parent strand. The almost infinite



variety observable in the diagrams of the present and of an earlier paper ((5), Text-Figs. 2 and 3, pp. 431, 433) in the length and course of these branchlets is the result chiefly of three factors. The level at which the protoxylem destined to a sporangiophore becomes individualized, and the extent of adjustment necessary for it to reach the radius of that sporangiophore determine, between them, the angle formed by the parent strand, and its branchlet, while the first factor and the amount of metaxylem developed in a lateral direction at the level at which the protoxylem leaves the stele between them determine the length of the branchlet visible in the diagram. The course of the protoxylem is, of course, established before the metaxylem is differentiated, and we may regard its divergence, whether in or outside the stele, as a continuous ontogenetic process.

The methods of adjustment of the variable and irregular axial protoxylem-system to the task of contributing the protoxylem to the traces of the relatively regularly disposed sporangiophores, usually alternating in successive whorls, may then be summarized under four heads: (1) anastomosis of the axial strands of protoxylem; (2) swerving of the unbranched axial strands of protoxylem; (3) divergence of the protoxylem while passing through the bundle; and (4) the pursuing by the whole trace of an oblique course through the cortex. Actually, all the cones studied show varying combinations of these methods of adjustment; and even for the supplying of a protoxylem to a single sporangiophore several of them may be, and frequently are, combined. This is, naturally, especially commonly the case for (3) and (4). Either, or both, (3) and (4), may be associated with anastomosis *or* swerving of unbranched axial strands, where neither of the last two processes has sufficed to bring the axial protoxylem opposite to the sporangiophore that it supplies. On the other hand, anastomosis and swerving of unbranched strands are alternative methods, though examples of both occur in all the cones studied. Small as is the cone of *E. variegatum*, the first three methods of adjustment are found in it. The narrowness of the stele of this species causes these adjustments to suffice, and no signs of divergence of traces in the cortex were observed in the three cones studied. In all the other species all four processes are combined in varying proportions. Thus, while as already observed, the cone of *E. arvense* shows the most numerous examples of the swerving of unbranched axial strands of protoxylem (Fig. 1), occasional anastomoses of the axial protoxylem occur in it, and intrastelar divergence of the protoxylem is common. Usually the width of the bundles is sufficient to allow of the necessary adjustment within the stele—at least in the cone shown in Fig. 1—and there are relatively few examples of traces passing obliquely through the cortex. In cones of this species with more extensive tracts of parenchyma, and less freely anastomotic bundles, a divergent course of the traces is somewhat commoner. In *E. silvaticum*, *E. limosum* and *E. debile*

(cf. Text-Figs. 2 and 3, pp. 431 and 433 of (5); and Fig. 5 of the present paper), but especially in the first of these, anastomosis of the axial strands of protoxylem is a factor of some importance, though divergence of the traces is common too. Since the bundles in these species are often very narrow, there is relatively less intrastelar divergence of the departing protoxylem. In *E. palustre* and *E. hyemale* (Figs. 3 and 4) all four modes of adjustment are found, though swerving of unbranched main strands of protoxylem is relatively rare. In *E. giganteum* (Fig. 6) both branching and swerving of unbranched axial strands of protoxylem are less common than an oblique course of the departing protoxylem within the bundle and of the trace through the cortex. This is partly due to the fact that, whereas in most species the strands of protoxylem of the cone tend to be equal to, or slightly more numerous than, the sporangiophores, in *E. giganteum* they are often slightly less numerous than the latter. Consequently, a single strand has not infrequently to give off protoxylem to sporangiophores lying on either side of itself. Finally, in the large cones of *E. maximum*, with their irregular vascular systems, examples of all four modes of adjustment can be observed (Figs. 2 and 2*a*), though, at any rate in the cone studied, anastomosis of the protoxylem-system is rare, and owing to the narrowness of many of the bundles, the amount of adjustment effected within the stele is often slight compared with the extrastelar adjustment.

It was suggested in 1921 that the incomplete fusion of the axial strands of protoxylem—failure of approximated strands to fuse would have been a more correct expression—might be found to be a phenomenon associated with prematurely divided—more accurately, with bifascicular traces ((5), p. 454). This expectation has not been fulfilled. Cases do occur of sporangiophores, single in nature (i.e. not consisting of two concrescent members), receiving two strands of protoxylem from approximated bundles. But usually the two protoxylem-strands of a bifascicular trace are given off by the same bundle, and we may properly speak of a prematurely divided trace.

There seems to be throughout the cones studied a tendency for portions of the protoxylem-system to become disconnected from the main system. This tendency is manifested in several ways. Perhaps the commonest indication of it is for the protoxylem of the sporangiophore not to enter into connexion with that of the axis of the cone. Either the whole trace dies out in the cortex, or the incoming phloem joins on to the axial phloem, but the protoxylem, which constitutes the xylem-supply of the sporangiophores, dies out within the bundle, among the tracheides of the metaxylem, without joining up with the axial protoxylem (7). This form of discontinuity seems to occur chiefly in the lowest whorl of the cone and is more marked in *E. maximum* than in the other species studied. In the lowest whorl of the cone of which Figs. 2 and 2*a* represent recon-

structions of the stele no less than twenty-five out of thirty traces show discontinuity between the appendicular and axial protoxylem. This is by far the most striking example of this phenomenon yet observed. But examples of a similar discontinuity have been observed in *E. limosum* ((3), p. 248), in *E. silvaticum* ((5), p. 438) and recently in *E. palustre* and *E. hyemale*. In all these species the discontinuity occurs chiefly at the level of the lowest whorl of sporangiophores.

The strands of protoxylem which are occasionally found ending blindly in a downward direction are another indication of this tendency for the protoxylem to become dissociated. These detached strands of protoxylem are almost always so clearly in the line of development of one of the strands at a slightly lower level that their discontinuity is clearly due to a local failure of parenchymatous cells to develop as protoxylem. As these cells lose their contents and become lignified and thickened later, they resemble the metaxylem-tracheides—or, to talk more correctly, they become metaxylem. Such separated strands of protoxylem are most often found in *E. maximum* ((7), p. 603), but have been seen also in *E. silvaticum* ((5), p. 454).

In several species, but especially in *E. maximum* and *E. arvense*, detached elements of protoxylem occur occasionally, apart from those constituting the definite protoxylem-strands ((5), p. 446-7).<sup>1</sup> In *E. variegatum* this dissociation of the protoxylem system is carried yet further. Here scattered elements of protoxylem habitually occur inside the edge of the xylem of the axis of the cone. In the stele of which Fig. 7 represents a reconstruction it was possible to trace the main lines of a connected protoxylem-system. But in the other two cones of this species studied the protoxylem was so irregularly distributed that it was impossible, except at the very base of the cone, to establish the existence of a definite system of protoxylem. The transition from proto- to metaxylem is here so gradual that it is often difficult to draw a line between the two.

#### DISCUSSION.

In 1912 I suggested that the vascular system of the cone of *Equisetum* was derived from a siphonostele ((2), p. 699) and Miss Barratt subsequently came, on rather different grounds, to a similar conclusion ((1), p. 230-1). As a result of the present investigations and of a reconsideration of the evidence an alternative and very different hypothesis suggests itself. It may be that in the cone the stele was primitively a circle of separate bundles that pursued an uninterrupted vertical course.

The evidence from the fossil cones supports this view. Unfortunately none of the older bractless cones, such as those of *Asterocalamites* and *Pothocites*, which seem to be closest to the strobili of *Equisetum*, has been

<sup>1</sup> The same is true of the elements of the metaxylem.

found with internal structure preserved. But in the Upper Carboniferous cones, *Calamostachys* and *Palaeostachys* the bundles seem, where the internal structure is known, to have run vertically as isolated strands—though they were sometimes double in nature or grouped in pairs (13), (14)—except for occasional anastomosis, apparently correlated to a change in number of the sporangiophores.<sup>1</sup> If this was so in cones in which the whorls of superposed sporangiophores were separated by whorls of bracts alternating amongst themselves, then, *a fortiori*, should we expect to find a similar unbranched course of the bundles in cones, such as those of *Asterocalamites* and *Pothocites*, which were either composed solely of whorls of superposed sporangiophores or of such whorls occasionally interrupted by leafy verticils.<sup>2</sup>

We know, too, that superposition of the sporangiophores gave way in the ancestors of recent Equisetaceae to alternation, probably towards the end of the Palaeozoic era. For in the genus *Equisetites*, of which some Tertiary species are practically indistinguishable from existing species of *Equisetum*, the Upper Carboniferous *E. Hemingwayi* Kidst. shows definite alternation of the sporangiophores ((18), p. 262, Fig. 57); while alternation does not yet seem to be fully established in certain specimens of *E. Muensteri* Stbg. from the older Mesozoic rocks of Sweden and Greenland.<sup>3</sup> It seems not unlikely that, as an adjustment to their task of supplying traces to alternating sporangiophores, the vascular bundles of the axis of the cone became, to a certain extent, anastomotic. This change from continuous bundles to bundles alternating at the nodes is found when we pass from *Asterocalamites* with its whorls of superposed, to *Calamites* with its whorls of alternating leaves. And we may note, in passing, that the same alternation of the bundles occurs in the vegetative axes of *Equisetum*, which bear whorls of alternating leaves. If, in the cones of the earlier Equisetaceae, the anastomosis of the bundles, resulting as an adjustment to the alternation of the sporangiophores, were localized to the neighbourhood of the pseudo-nodes, as it is in the axis to that of the nodes, this would tend to give the impression of a local siphonostele, such as is sometimes seen at this level, especially in the cone of *E. arvense*.

In a series of papers dealing with the cones of *E. arvense*, *E. palustre*, *E. limosum*, *E. giganteum*, *E. hyemale*, *E. sylvaticum*, *E. debile*, and *E.*

<sup>1</sup> It is possible that in *Calamostachys magnae-crucis* Browne the bundles alternated at the node (8).

<sup>2</sup> Though no case of siphonostely has been recorded from the Equisetales proper in *Calamophyton primaevum* Kräuse and Weyland, a possible Devonian forerunner of the phylum, in a single ill-preserved fragment of the vegetative axis, indications of what may perhaps prove to be a siphonostele have been found (15, p. 139).

<sup>3</sup> See (11) Halle's Figs. 12 and 13 of Pl. VIII and Figs. 12-14 of Pl. IX. Harris (12), p. 12 identifies these forms, provisionally described by Halle as *Equisetistachys suecicus* as cones of *Equisetites Muensteri* Stbg.

*variegatum* I have concluded that the irregularity of the network of strands found in the cones of certain species (and most marked in that of *E. maximum*) arose by the persistence of tracts of parenchyma separating the bundles. These tracts were held to have been originally, phylogenetically speaking, bridged transversely by the fusion of the bundles near the level of insertion of the sporangiophores, and therefore to have extended at first only from the level of one whorl to that of the next. Thus, loss of anastomosis owing to poor development of the vascular system, was held to be the principal factor in the evolution of the more irregular types of stele. Under the influence of belief in the siphonostelic origin of the stele these tracts of parenchyma were called meshes and were supposed to have arisen in a pre-existing siphonostele at points vertically over, but not directly above, traces that had departed, where the flow of water would naturally be diminished. If it be held that the steles showing an irregular network of poorly-branched bundles, have been derived by vascular reduction from forms with a more highly anastomotic vascular system (at least as regards the metaxylem), then it seems to me that the evolution of the cone has probably followed more or less the lines laid down in further detail in those papers, to which the reader is referred.<sup>1</sup> If we abandon the siphonostelic origin of the stele, we shall, instead of persistent and extending meshes, speak of bundles which have failed to anastomose, thus leaving the parenchymatous ground-tissue to form an irregular network, instead of breaking it up into separate bands between the bundles alternating (as the latter are held to have alternated before the loss of anastomosis) from one pseudo-internode to the next. The process, though described in different terms, would be visualized as being, in fact, similar, once the stage of regularly alternating strands had been supposed to be attained.

But if the anastomosis of the bundles of the cone was originally an adjustment to the change from continuous bundles to bundles alternating at the pseudo-nodes it was probably, at least at first, tentative; it may not have affected the protoxylem to the same extent as the metaxylem; and there would be no sufficient reason for assuming that a regularly anastomotic vascular system was ever established. If this be so, then it follows that the steles of certain cones showing other adaptations than anastomosis to the supplying of traces to alternating sporangiophores—showing, for instance, an obliquely outward course of the trace through the cortex, or divergence of the protoxylem within the bundle, or swerving of the strand of protoxylem—are not, on account of these adaptations necessarily modified from more highly anastomotic steles. All these kinds of adjustment may be, so to speak, coeval with the adjustment of anastomosis; on the other hand, they may in certain cases have been evolved as the

<sup>1</sup> (2), pp. 670-3; (3), pp. 235-8; (4), pp. 240-9; (5), pp. 430-6 and 450-2.

result of a loss of anastomosis. In fact, if the doubts outlined above are justified, instead of visualizing the evolution of the stele of the cone within the genus as broadly speaking a process of reduction,<sup>1</sup> we find ourselves confronted with a much more complicated problem or series of problems, since in each species, or group of closely related species, we remain uncertain which are the forms that have the more primitive stele and cannot even feel sure whether a relative rarity of anastomosis is primitive or due to reduction. It may be the former in one species and the latter in another.

Certainly, in the cone of *E. arvense* the failure of the protoxylem to anastomose as freely as the metaxylem, combined with the obvious tendency of the latter to develop in a lateral direction, does suggest an increase, within the genus, of anastomosis of the bundles.<sup>2</sup>

On the other hand, except on the view that they are vestiges of a former siphonostele, it is difficult to see any explanation of the occurrence of those wide sweeps of metaxylem, found occasionally in *E. hyemale*, *E. palustre* and *E. maximum*, but more especially in *E. giganteum* and *E. arvense*, extending from the level of one whorl to that of the next and involving more than the equivalent of one bundle. Can we, if we reject the siphonostelic origin of the central cylinder, account for these tracts of vascular tissue by supposing that the acquisition of anastomosis of the bundles has led to a vigorous development of metaxylem in the neighbourhood of the branching and fusion of the bundles and that, with the shortening of the interval between the whorls, this metaxylem has come to extend over the whole of a pseudo-internode? Any one familiar with a young cone of *Equisetum* will have noticed how effective is the protection afforded to the developing sporangia by the close imbrication of the heads of the sporangiophores. These form a regular mosaic, characteristic of all the cones of the genus during their immaturity i.e. whilst the vascular elements are being differentiated. The closeness of the imbrication is only made possible by the combined effects of the alternation of the sporangiophores of successive whorls and of the shortening of the pseudo-internodes, a shortening probably correlated to the diminution in size of the Equisetaceae as we pass through the Mesozoic and Tertiary ages.<sup>3</sup> Possibly, too, the tendency already noted for the metaxylem to develop laterally rather than radially may have facilitated the formation of these bands in *E. arvense* and *E. maximum*, though not in *E. giganteum*, where the metaxylem is well developed radially.

<sup>1</sup> I had always contemplated the occurrence, combined with a general reduction of the vascular system of the cone, of certain tendencies leading to a slight increase, in some species, of vascular tissue. Cf. (3), pp. 237-8; (4), pp. 248-9.

<sup>2</sup> Curiously enough, this development of the metaxylem laterally at the expense of its radial extent is characteristic also of *E. maximum*, where it does not seem to have led to any marked increase of the branching of the bundles.

<sup>3</sup> For this reduction in size, see (17), p. 73.

But, even if the protoxylem of the cone was never as freely anastomotic as the metaxylem, there are indications that it has suffered reduction. It has been argued that the arrangement and mode of development of the strands of protoxylem are features that would be least affected and longest retained in any process of reduction ((1), p. 223). But in the cones of *Equisetum* these strands are too slender to serve a mechanical purpose; as their function is the conduction of water to the developing sporangia, it is quite likely that with a reduction in the number of sporangiophores, such as seems to have occurred in the ancestors of *Equisetum*, we might get a reduction of the protoxylem. Throughout the genus the bundles of the vegetative axes anastomose regularly at or near the insertion of the whorls of leaves,<sup>1</sup> but a similar forking and fusion of the protoxylem, though it too occurs (aerial branches of *E. giganteum*, cone-bearing branches of *E. silvaticum* and sometimes, at least, of *E. arvense*) is not general. Sometimes the protoxylem forks at the node, but one or both forks may, or may not, die out (*E. debile*), or this dying out may be the rule (cone-bearing branches of *E. maximum*), while in other cases (*E. hyemale*, sterile shoots of *E. arvense*) the protoxylem strand passes out unbranched in its entirety into a leaf-trace, so that the protoxylem system is discontinuous in successive internodes ((16), p. 267; (1), p. 229; (6), p. 459-63). Here we get an analogy for a greater loss of anastomosis by the protoxylem than by the metaxylem.

In the cone of *E. maximum* the protoxylem shows various signs of reduction. Parenchymatous cells generally, perhaps constantly intervene between it and the metaxylem; certain elements, obviously in the line of continuation of the protoxylem, seem to be converted in tracheides so late that they resemble the surrounding metaxylem and cause a break in the protoxylem-strand. Frequently in this species and in *E. arvense* and occasionally in other species, isolated groups of protoxylem, of too small a vertical extent to be shown in the diagrams, are found. In *E. variegatum* the protoxylem elements are sometimes so irregularly scattered that it may be impossible, except at the base of the cone, to determine the lines of a main protoxylem-system. Attention has already been drawn (7) to the fact that in certain cases the protoxylem of the sporangiophores is not in continuity with that of the axis of the cone. Such a condition is by no means uncommon in cones of *E. maximum* and may, indeed, be called common at the base of the cones of this species. In cones of *E. maximum* every gradation between the failure of the trace of the sporangiophore to enter the axis at all and the dying out of the incoming protoxylem in the axial metaxylem and in close proximity to the axial protoxylem has been observed (7). That such conditions indicate modification by reduction can hardly be doubted, but perhaps an even more striking indication of this is

<sup>1</sup> For the very rare exceptions, see (5), pp. 440-1, and (9), p. 54.

afforded by the loss of verticillation, so exceptional among the Equisetales, shown by many of the larger cones of *E. maximum*. The loss of verticillation is more marked for the points of insertion of the traces on the stele than for those of the sporangiophores on the axis.<sup>1</sup> In the (4-7) lowest whorls of the cone, though there is a great deal of variation between the levels of departure of traces belonging to the same whorl, still, the difference is not generally sufficient to obscure the distinction, in a reconstruction of the stele, between traces of sporangiophores belonging to different whorls. But in this species it is frequently impossible, from a study of the stele alone, to determine to which whorl a given trace belongs (see Figs. 2 and 3, and (3), Pl. XII and XIII). In larger cones of *E. maximum* the verticillation of the sporangiophores themselves is often lost.<sup>2</sup>

To conclude, the evidence does not all point in one direction: the frequent absence of correlation between meta- and protoxylem, the rarity of anastomosis, and especially of fusion, in the latter system, and the evidence from the fossil forms, suggest the possibility that the central cylinder originally consisted of a circle of separate unbranched strands; and that, where anastomosis occurs, it is one of several methods of adaption to the task of supplying traces to whorls of alternating sporangiophores. On the other hand, the occasional wide bands of metaxylem, extending from the level of insertion of one whorl to that of the next, suggest a primitively siphonostelic vascular system; while the numerous indications of reduction of vascular tissue—particularly of the protoxylem—suggest that the more highly anastomotic steles are the more primitive and that the protoxylem, which is so much less freely anastomotic than the metaxylem, is also more modified by reduction than the latter. The question remains open and we can only say that the probability is that in certain forms, such as *E. maximum* and *E. variegatum*, there seems to have been some loss of anastomosis, especially of the protoxylem, even if a regularly anastomotic vascular system was not found in any ancestor of the existing species.

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<sup>1</sup> That this should be so is rendered possible by the fact that the level of departure of the traces from the stele varies even for adjacent traces of the same whorl (cf. (3), p. 243).

<sup>2</sup> (10), pp. 1106-1107 and Fig. 1090, p. 1110; (3), p. 238. The close imbrication of the sporangiophores does not appear to be lost, even when their alternation is irregular.



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# Teratological Studies in the Tubiflorae.

## I. Abnormalities in the Flowers of *Antirrhinum majus* L.

BY

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With eleven Figures in the Text

DURING the late summer of 1930 a plant of *Antirrhinum majus* bearing abnormal flowers was observed in a garden at New Barnet: the flowers attracted attention because they appeared to be double, but examination showed that the appearance was due to petaloid structures connected with the stamens. In June, 1931, three inflorescences of the same species were collected at Slough; they seemed to show similar abnormalities. A preliminary examination of one or two flowers indicated that a detailed study of all the blossoms was desirable. All the flowers of the four inflorescences, with the exception of some of the smallest buds, were therefore examined in detail: abnormalities were found in the gynaeceum, androeceum, and corolla, as well as irregularities in the flower as a whole. Some of these anomalies have been recorded by other investigators, some appear not to have been described previously.

In the descriptions which follow, the flowers are referred to by numbers, in the order of their position on the raceme. The New Barnet inflorescence is referred to as B, the Slough racemes as S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> respectively. Branches occurring on the inflorescence are referred to as Br. 1, &c., in the order in which they occur. Thus B/2 designates the second oldest flower on the New Barnet inflorescence; S<sub>3</sub>/Br<sub>2</sub>/1 refers to the oldest flower on the second oldest branch of the third Slough inflorescence.

### 1. *Abnormalities in the Flower.*

A. *Synanthy.* One instance of synanthy occurred, in S<sub>1</sub>/Br<sub>1</sub>/1. The shoot S<sub>1</sub>/Br<sub>1</sub> sprang from the base of the raceme; it had an axis about an inch long, terminated by a whorl of three bracts. In the axil of one of the bracts (Fig. 1, *br.* 3) was a short shoot bearing a few minute buds (*fs*); from the same whorl there arose a bud some 9 mm. long, in which the

corolla was almost enclosed in the calyx; compared with other buds of the same length, this one was very stout. It had 8 sepals; of these the posterior one (*a*) was larger than any of the others, except the one occupy-

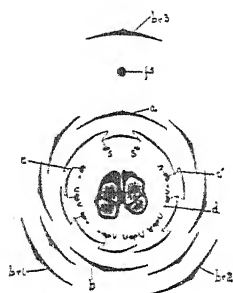


FIG. 1.

FIG. 1. Diagram of St/Bri. *a*, larger, posterior sepal. *b*, larger, almost anterior sepal. *br1*, *br2*, bracts of flower, *br3*, bract at base of flowering shoot. *c*, *c'*, clefts between anterior and posterior parts of corolla. *fs*, undeveloped flowering shoot. *s*, staminode.

The dots and U-shaped structures attached to the stamens represent staminal appendages: of these *d* is adnate to the corolla.

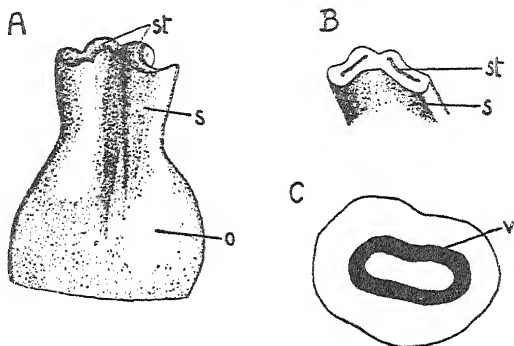


FIG. 2.

Fig. 2. St/Bri/1. A. Gynaecium ( $\times 2$ ). B. Style and stigmas from above ( $\times c. 6$ ). C. Diagrammatic T. S. Pedicel ( $\times c. 18$ ). *o*, ovary. *s*, style. *st*, stigmas. *v*, area occupied by vascular bundles.

ing a position slightly left of the anterior (*b*). The corolla segments numbered ten; there was a small posterior lobe, and on either side adjacent to it, a relatively large segment suggesting the two postero-lateral lobes of the normal corolla; the seven more anterior segments were rather small, and it was not found possible to ascertain if one or more had a saccate base; the somewhat deeper clefts which in a normal corolla separate the upper and lower lips, appeared in this flower to be present at *c* and *c'*. The androeceum consisted of eight stamens with the appendages to be referred to subsequently, and two minute staminodes (*s*) posteriorly. The gynaecium had a short style (Fig. 2 A), flattened, and with the short axis in the antero-posterior plane; there was a groove down the middle of each broad face; the style terminated abruptly in a flat top, which presumably represented the stigmas (*st*). A transverse section of the ovary (Fig. 1) showed that it consisted of four loculi, the two on the right separated from those on the left by a strip of tissue, grooved posteriorly and anteriorly. It will be noticed that the placentation is axile in relation to the right and left halves of the ovary, not in reference to the ovary as a whole; each half shows an ovary structure typical of the normal flower.

There is little doubt that this singular bud arose as the result of fusion of the rudiments of two flowers. The number of parts in the various whorls supports this view: the ovary is evidently produced by the lateral fusion of two ovaries. It is true that the sepals number eight and not ten,

but the larger size of *a* and *b* suggests that these each consist of two fused sepals. Further, the flower has two bracts (Fig. 1, *br*<sub>1</sub>, *br*<sub>2</sub>). Additional evidence in support of the view that the bud consisted of two fused flowers was obtained from a transverse section of the pedicel (Fig. 2C), which showed this to be flattened; its vascular tissue formed an oval area, not a circular one as in the normal pedicel.

Synanthly appears to be not uncommon in the Scrophulariaceae, and Masters (10) cites six genera in which it has been recorded, including *Antirrhinum*. Worsdell (16), however, carefully differentiates between synanthly and fasciation, and would not regard as synanthous, as does Masters, such abnormalities as the campanulate terminal flower of *Digitalis*: it is not improbable that a number of cases of synanthous flowers of *Antirrhinum*, cited by older authors, are actually due to fasciation in Worsdell's sense. The abnormality which is most nearly allied to that just described is recorded by Wigand (15) in *Pedicularis sylvatica*: the two most distal flowers on the inflorescence were fused, forming a single peloric flower; this appears to be a rare condition. This flower differed from the synanthous *Antirrhinum* bud in its terminal position, in the possession of two separate ovaries, and the eight members in the other whorls; there were two bracts. It is thought that the *Antirrhinum* bud would not have shown pelory in its corolla when it expanded into the flower, but it was not possible to be sure of this in so small a bud. Chavannes (5) figures a peloric flower of *Antirrhinum majus* formed by the union of two flowers: this appears to have a corolla with seven lobes, and there are six stamens; details of the calyx and ovary are lacking.

In S<sub>2</sub>/Br<sub>2</sub> the axis was flattened, with a well marked median groove on the flattened faces. The branch bore two flowers, arising from the axil of a single bract at the apex of the flattened axis: this bract was, without doubt, a double structure; it had two apices in place of the normal single one, and was traversed by two vascular strands, one running to each apex. Sections of the flattened axis showed that near the base the vascular tissue was in two rings, only slightly fused, while distally it formed an ellipse. In this case, then, concrescence had occurred in the axis of the branch, up to and including the bracts, in contrast with S<sub>1</sub>/Br<sub>1</sub>/1, in which concrescence began immediately above the bracts. These two anomalies appear to be closely related, and emphasize the difficulty expressed by Worsdell ((16) pp. 237-8) of differentiating between synanthly and fasciation.

Fasciation occurred at the base of the inflorescence S<sub>2</sub>. It continued until three branches were given off at the same point; of these, two had normal axes, the third was the branch just described.

B. *Alteration in the plane of symmetry of the flower.* Two flowers were noted in which the plane of symmetry deviated from the normal one. In B/3, which was not actually a flower of the raceme but occurred on

a short lateral branch, this branch bore a small leaf, and above this two opposite leaves, one (Fig. 5 *b*) subtending the flower B/3, the other larger leaf (1) subtending a minute shoot (*as*). The interpretation placed on the

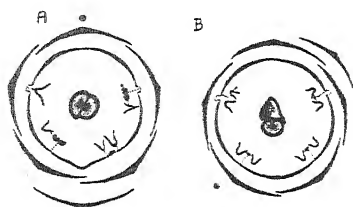


FIG. 3. Floral diagrams of: A. B/1. B. B/3. The dots and U-shaped structures attached to the stamens represent staminal appendages.

structures is that B/3 is the first flower of the undeveloped shoot (*as*), *b* is its bract. If this be so then the normally left antero-lateral sepal is posterior; in any case, if the flower be orientated by its bract, the right postero-lateral petal is anterior (Fig. 3 B). No trace of torsion in the pedicel could be seen.

In B/1 (Fig. 3 A) the septum separating the two loculi was oblique, as in the Solanaceae. It is interesting to note that in this flower the left postero-lateral stamen was sterile, which accords with Robyns's (13) statement for the Solanaceae that the symmetry of the flower coincides with the obliquity of the carpels. One must not, of course, attach too much importance to this feature, as it has only been found in a single flower.

## 2. Abnormalities in Calyx and Corolla.

*A. Calyx.* The aestivation of the sepals was very uniform. In most cases the odd sepal was internal; in three flowers this member was external, and in three instances internal on one side and external on the other. With one exception these irregularities occurred in flowers of the B series.

How far the arrangement in which the odd sepal is internal holds for the Scrophulariaceae is a matter for speculation; in published floral diagrams it is shown sometimes as external and sometimes as internal. Its internal position in flowers of the S series was, however, remarkably constant.

Variations in the corolla were of sporadic occurrence, and their possible significance difficult to assess.

*B. Pelory in the corolla.* None of the flowers was peloric, but an approach to this condition was observed in the corollas of one or two of the B flowers. Thus in B/2 the posterior petals were not so large as usual, so that the upper and lower lips approximated in size. The same condition was, perhaps, even more marked in B/4 (Fig. 4 D): in all cases, however, the zygomorphic nature of the corolla was readily discernable on the most casual examination.

*C. Fission of the corolla.* In B/4 the corolla was cleft for about half its length between the left postero-lateral and antero-lateral petals (Fig. 4 D): between the right postero-lateral and antero-lateral segments a cleft occurred extending to within 5 mm. of the base.

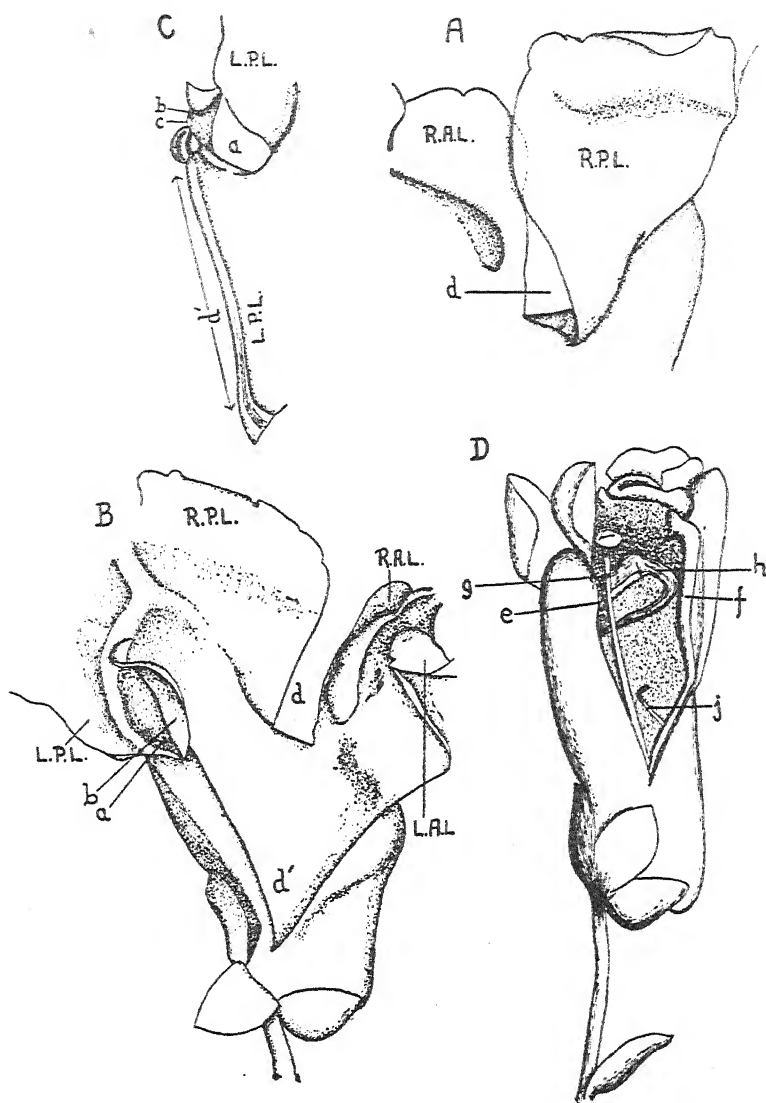


FIG. 4. A-C. Corolla of *S<sub>2</sub>/Bri/3*. A. Right-hand side of corolla from outside, showing normal cleft between right antero-lateral and right postero-lateral petals. B. Corolla from left-hand side, showing deep cleft between left antero-lateral and left postero-lateral petals, and normal cleft between right antero-lateral and right postero-lateral petals. C. Left postero-lateral stamen. D. Flower B/4, showing deep cleft between left antero-lateral and left postero-lateral petals: note approach to pelory in corolla. All  $\times 2$ .

L.A.L. Left antero-lateral petal. L.P.L. Left postero-lateral petal. R.A.L. Right antero-lateral petal. R.P.L. Right postero-lateral petal. *a*. Space between left postero-lateral petal and *b*. *b*. Petaloid flap. *c*. Minute anther-like portion of left postero-lateral stamen. *d*. Cleft between right antero-lateral and right postero-lateral petals. *d'*. Cleft between left antero-lateral and left postero-lateral petals. *e*. Flap of tissue, possibly petaloid. *f*. Hooded appendage of left postero-lateral stamen. *g*. Right antero-lateral stamen. *h*. Hooded appendage of right antero-lateral stamen. *j*. Filamentous appendage of left antero-lateral stamen.

In S2/Br1/3 the cleft between the postero- and antero-lateral segments of the right side was normal, but that on the left extended nearly to the base (Fig. 4 B, *d'*); from the top of its posterior margin there sprang a small petaloid flap (*b*); the space (*a*) between the left postero-lateral petal and the flap was similar in appearance to the hinge normally occurring between the postero-lateral and antero-lateral segments, and suggests the possibility that the cleft (*d'*) was actually in the left antero-lateral segment of the corolla. However, the left postero-lateral stamen was adnate to the corolla, and lay along the posterior margin of the cleft (Fig. 4 C), which favours the view that the cleft was merely an extension of the normal cleft which usually occupies this position. In favour of this view it is possible to regard the flap (*b*) as a petaloid outgrowth from the top of the stamen, but there is no proof that this is the case.

These anomalies appear to call for little comment; they are merely extensions of the fairly deep clefts which normally separate the upper and lower lips of the corolla. The other instances of partial dialysis of the corolla are more unusual. In S2/Br2/2 the separation of the right postero-lateral and right antero-lateral segments extended nearly to the base of the corolla, but there was also a deep incision between the anterior and left antero-lateral petals. In S2/7 the corolla, which was rather thinner than usual, had an anterior petal separated almost to the base from the antero-lateral members. The limb of the anterior petal was rather narrow, but possessed nevertheless an apparently normal saccate base.

Masters (10) records separation of the petals in this species: Penzig (12) notes that it is more frequent between the posterior petals and less frequent in the positions described above.

In S2/5 the corolla had a contorted appearance; this appears to have been brought about by growth of different areas proceeding at different rates: allowing for the twisted appearance, the corolla had the structure of that of normal flowers.

### 3. *Abnormalities in the Androeceum.*

A. *Enation*. It was this feature which first drew attention to the flowers: of all the anomalies described this occurred most frequently. In what may be regarded as its most complete form (Fig. 6, B and C) a stamen consisted of a complex of three members, a central fertile segment, and two lateral, sterile ones. The central member resembled in every way a normal stamen of the species, except that its filament was often somewhat flattened; the lateral segments consisted of a limb in which the margins were often bent towards one another, forming a gutter-shaped or tubular structure, and a broad, flap-like apex, sometimes bent back over the limb. The flap and the upper part of the limb were usually coloured like the



corolla, and it was these petaloid structures, protruding through the mouth of the corolla, which gave the appearance of a double flower (Fig. 5). These lateral appendages were united to the fertile segment, which is subsequently referred to as the stamen, and arose very close to the base, and usually somewhat on the outer side. In no case did they arise from the corolla, and even when adnate to the corolla had their origin at the bases of the stamens. Sometimes both appendages were well developed, sometimes only one, and it was possible to trace all stages between the hooded, petaloid structure just described, through somewhat spatulate bodies (Fig. 6, D and K), and long, filiform appendages (M) to minute teeth (L and N). Sometimes one of the lateral members was absent (H and I), or both members were missing, and the only trace of abnormality appeared in the flattened filament of the stamen; finally the flattening of the filament was not seen and the stamen appeared to be normal.

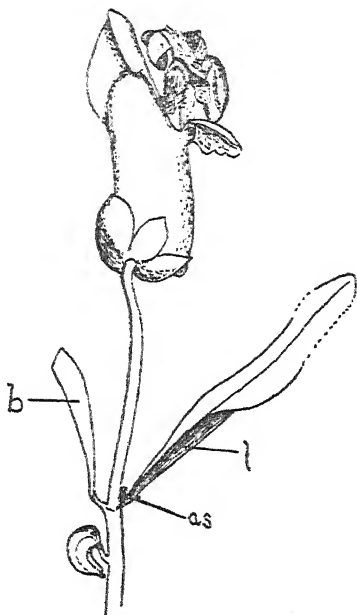
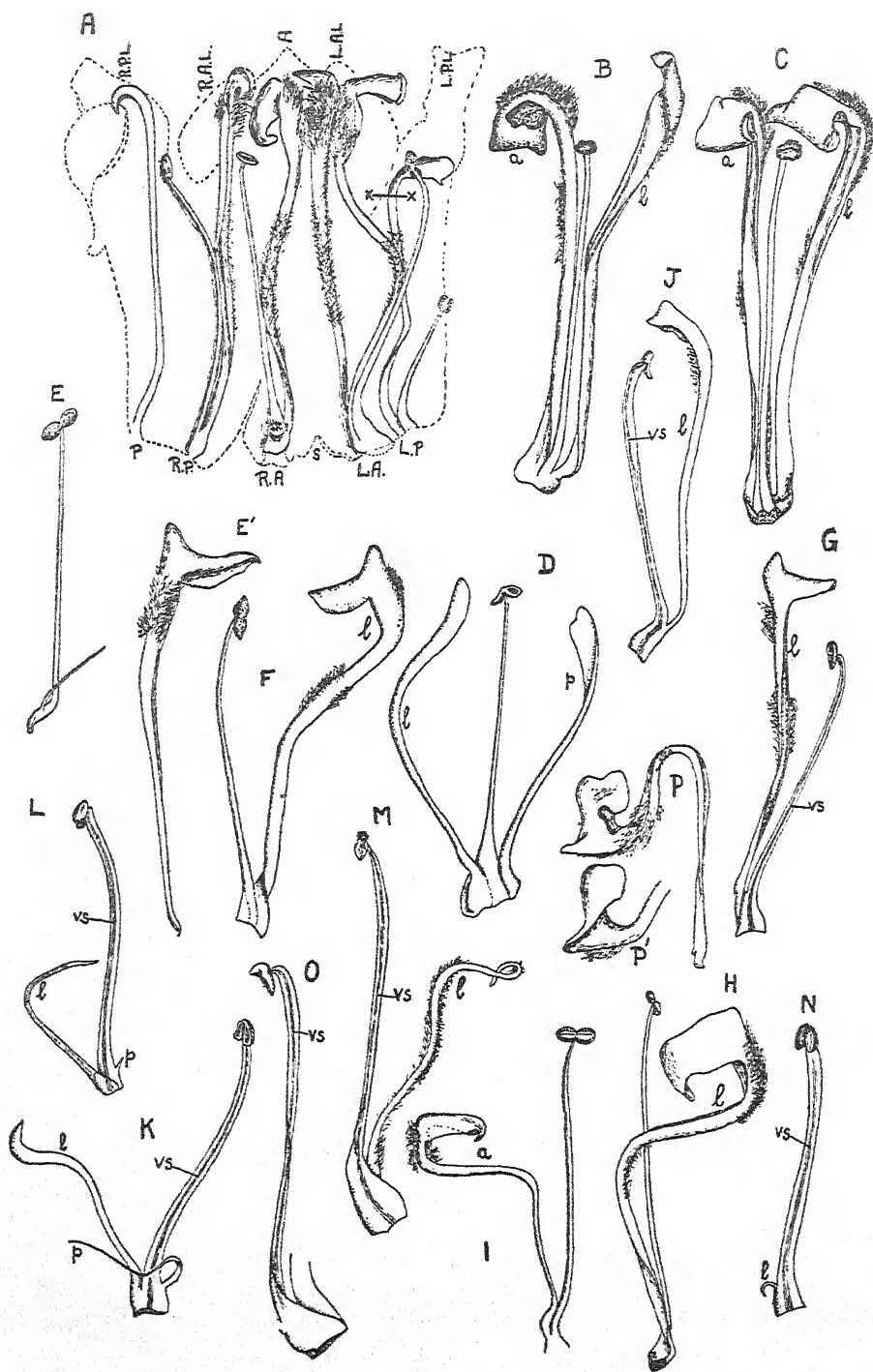


FIG. 5. Flower B/3. *as.* Axillary shoot.  
*b.* Bract of flower. *l.* Leaf. ( $\times 1$ .)

That the appendages were part of the staminal whorl was further demonstrated by an examination of young flower buds, in which the anthers were almost fully developed, but the filaments still short. Close to the base of the filament, and somewhat on the outer side were situated paired lateral appendages, in the form of minute teeth (Fig. 7). Examination of successively older buds showed that these teeth developed into the appendages.

In expanded flowers the appendages were sometimes situated so near the base of the flowers that it was difficult to be sure of their exact origin; if, however, such a stamen were carefully removed, the appendages always came away with it. Examination of the vascular system of the staminal complex showed that there was a single large vascular strand, and that this always ran up the fertile segment; with one or two exceptions its course could always be followed with ease. If the whole complex be sterile it is reasonable to conclude that the segment which contains the large vascular strand represents the stamen; this was always the central segment when three were present. The presence of the main vascular strand in the staminal segment seems to indicate the subsidiary nature of the appendages.

*B. Cohesion and adnation.* Cohesion between the stamens and their



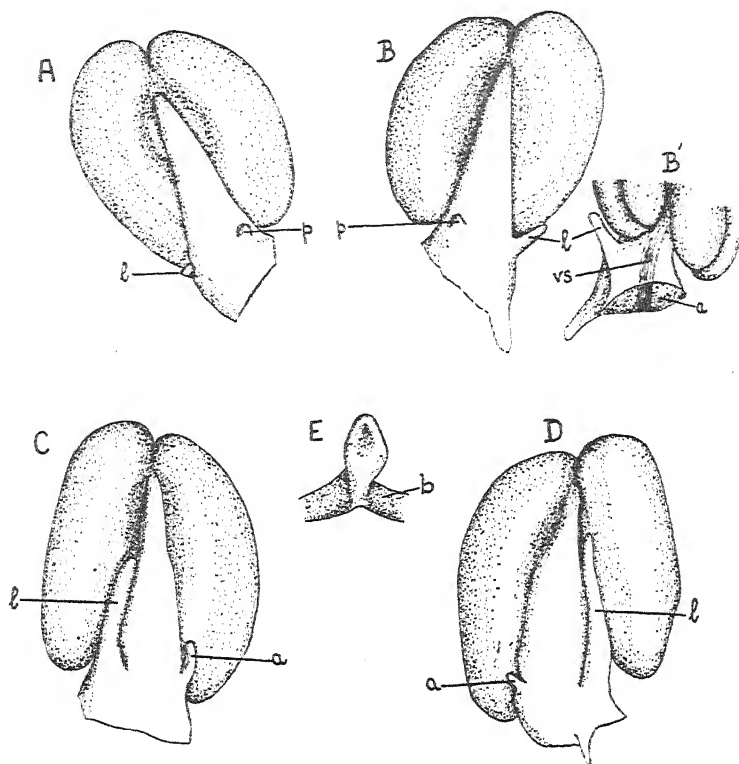


FIG. 7. Stamens of *St/17*, a small bud about 5 mm. long. A, R.P. B, L.P. B', Base of L.P. Note vascular strand. C, L.A. D, R.A. E, P. B' and E show the inner side of the stamen, the remainder the outer side. All  $\times c. 18$ . *a*, area of attachment to corolla. *b*, base of corolla. Other lettering as in Fig. 6.

appendages occurred fairly frequently, although normally the two structures were separate except at the base. Where cohesion occurred the appendage

FIG. 6. Stamens selected from various flowers, to show variation in the staminal appendages. All  $\times 2$ . A, B/2. Androecium: corolla indicated by broken lines. Corolla was opened by a posterior slit. L.P.L. is adnate to the corolla up to the line *xx*. B, B/3, L.A., and C, B/3, R.A., both have two hooded appendages. In C the gutter-shaped limbs of the appendages are seen. D, B/5, L.P. Appendages well developed, but not hooded. E, B/1, R.P. Stamen with filamentous appendage: it also bore a hooded appendage, E'. F, B/5, R.P., G, B/6, L.P., and H, B/1, L.A. Stamens with a single but well developed hooded appendage. Note course of main vascular strand in G. I, B/4, R.A. Stamen bears only one appendage, and this less well developed than in F-H. J, B/6, R.P. As I, but filament of stamen flattened. K, *St/3*, L.P. Stamen with two appendages, of which one is fairly well developed, the other filamentous. L, *St/3*, R.P. Stamen with two appendages, one filamentous, the other a small tooth. M, B/6, L.A. Stamen bears a single, filamentous appendage. N, *St/4*, L.P. Stamen has a single, very small appendage. O, B/5, L.A. Stamen with flattened filament: appendages omitted. P. Occupied position of L.P. stamen in B/1: probably the appendage of an undeveloped stamen. P', section through the hooded end of P. L.A. Left antero-lateral stamen. L.R. Left postero-lateral stamen. P. Posterior staminode. R.A. Right antero-lateral stamen. R.P. Right postero-lateral stamen. A. Anterior petal. L.A.L. Left antero-lateral petal. L.P.L. Left postero-lateral petal. R.A.L. Right antero-lateral petal. R.P.L. Right postero-lateral petal. *a*, appendage on anterior side of stamen. *l*, appendage on lateral side of stamen. *p*, appendage on posterior side of stamen. *s*, saccate base of anterior petal. *vs*, main vascular strand.

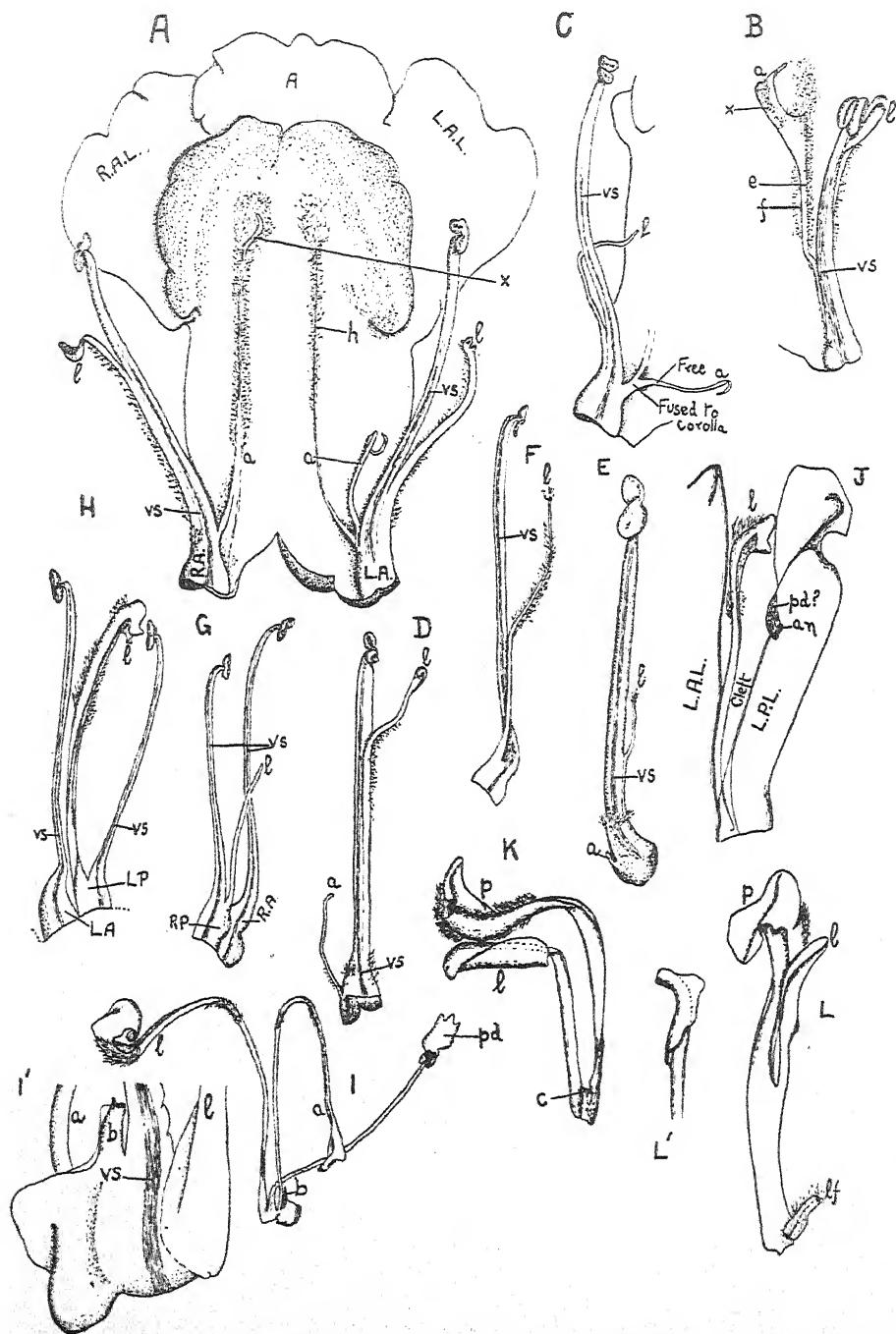


FIG. 8. Stamens selected from various flowers to illustrate unusual types. Unless otherwise stated, all  $\times 2$ . A, *Si/2*, anterior half of corolla with the two antero-lateral stamens: anterior

sometimes seemed to arise from some distance up the filament (Fig. 8 E, F), but there is little doubt that this was due merely to a more intimate union than usual. All stages between free appendages, and cohesion of appendages except at the tips (Fig. 8 A and D), were found. Occasionally both lateral members were fused to the stamen for part of their length, but it was more usual for one, at least, to be free. In one case, B/7 or 8 (the corollas of these two flowers had fallen off and it was not possible to ascertain from which of the two pedicels each had come), the antero-lateral and the postero-lateral stamens of the right side were fused together by their bases (Fig. 8 G) to form a single group; those of the left side were in contact at the extreme base, but were free from one another (Fig. 8 H).

Not uncommonly the appendages were adnate to the corolla, either in their basal region (Fig. 8 C) or for the greater part of their length (Fig. 8 A). The coherent part of the anterior segment of the anterior stamens occupied the position normally occupied by the honey-guide, and was invested with a felt of hairs (Fig. 8 A).

In a few cases the stamen proper was adnate to the corolla, and in nearly all these cases sterility occurred; not all sterile stamens were, however, fused to the petals. Adnation occurred in the right postero-lateral stamen of B/2, which was fused throughout its length to the corolla. In B/4 this stamen was in a similar condition, and was sterile, and the left postero-lateral stamen of the same flower was also fused to the corolla, and in this region the corolla was cleft nearly to the base (Figs. 4 D and 8 J).

An analysis of the occurrence of certain anomalies of the androeceum is given in Table I.

It will be seen that where only one appendage is present on a stamen, this is almost always the lateral one. Further, instances of fusion between appendages and stamens invariably occurred in anterior members of the androeceum and nearly always affected the lateral appendage, more rarely the anterior one; where, however, appendages were adnate to the corolla,

segment of R.A., adnate to corolla except at tip: lateral appendage of R.A., concrescent to stamen for greater part of its length. B, St/6, L.A., showing lateral appendage partly concrescent with stamen, but free at tip, which is somewhat hooded: anterior appendage concrescent with stamen at base, adnate to corolla in middle and free at tip, which is petaloid. C, St/3, R.A., showing base of anterior appendage adnate to corolla, and lateral appendage concrescent with corolla for a short distance. D, St/3, L.A. E, St/9, L.A. F, B/6, R.A. All show concrescence of appendages with stamens. In D and E the filaments of the stamens are markedly flattened: compare with filaments in G. G, B/7 or 8, R.A. and R.P., showing fusion between the bases of the two stamens. H, B/7 or 8, showing stamens L.A. and L.P. close together but not fused. I, B/1, R.A., the stamen carries a petaloid flap above the anther. I', B/1, R.A., base, from outer side. x 10. J, B/4, L.P., stamen adnate to corolla, appendage is free for part of its length. K, B/3, L.P., no fertile stamen. The peg of tissue, *c*, possibly represents the undeveloped stamen. L, B/3, R.P., no fertile stamen: the leaf-like structure, *lf*, possibly represents the stamen. Note that the appendages are fused together in their proximal halves. L', B/3, top of lateral appendage of L, from outer side, showing hooded nature *an*, anther? *b*, small peg of tissue at base of stamen. *c*, small peg of tissue: undeveloped stamen? *e*, edge of appendage adnate to corolla. *f*, free edge of appendage. *h*, honey-guide. *lf*, leaf-like structure. *pl*, petaloid flap. *st*, stamen. *x*, free tip of appendage. Other lettering as in Fig. 6.

in nearly all cases the anterior ones were affected. It would seem that too many instances are recorded in the table for the distribution of these anomalies to be regarded as fortuitous; but their significance is obscure.

TABLE I.

*Distribution of Certain Anomalies in the Androeceum.*

	Posterior stamens.		Anterior stamens.		Total.
Adnation of stamen proper to corolla . . . . .	7		6		13
	Lateral appendages.	Posterior appendages.	Lateral appendages.	Anterior appendages.	
Single appendage present	18	0	8	3	29
Cohesion of appendages to stamen . . . . .	0	0	24	2	31 <sup>1</sup>
Fusion of appendages to corolla . . . . .	0	1	1	8	11 <sup>2</sup>

C. *Sterility and petaloidy.* Sterility of the stamens was rare and was found most frequently in flowers of the B series. In B/3 both postero-lateral members were sterile: that on the left (Fig. 8 K) consisted of two well-developed petaloid segments and between them a minute peg of tissue up which ran the main vascular strand; it would seem that this small median point is to be interpreted as the undeveloped stamen, bearing two well-developed lateral appendages. This explanation probably applies to the right postero-lateral stamen of the same flower, but this was more difficult to elucidate; it consisted of a limb, branching half way up into two petaloid bodies (Fig. 8 L); at the base of the limb and on its inner side was a small leaf-like structure (*lf*), which probably represented the stamen proper; no single, well-developed vascular strand was visible in the whole structure; there is little doubt that the limb represented the fused limbs of the two appendages. In the position of the left postero-lateral stamen of B/1 (Fig. 6 P) was a single well-developed petaloid member, which may have represented an appendage of the undeveloped stamen.

In B/1 the median member of the right antero-lateral stamen possessed anthers, and above this a petaloid flap occurred (Fig. 8 I, *pd.*), suggesting a broadening out of the distal end of the filament. This condition probably leads to cases like that of the left postero-lateral stamen of B/4, where the anther (Fig. 8 J, *an.*), like the filament, was adnate to the corolla, and small, if not abortive; above it was a structure (*pd?*), which probably corresponds to the petaloid flap of B/1. In the right postero-lateral member the filament was likewise adnate to the corolla and terminated in a flap of tissue (Fig. 11, L, R. P. L), without trace of a proper anther. In one instance

<sup>1</sup> In five instances cohesion of both appendages to the anterior stamens was noted.

<sup>2</sup> In one instance both appendages were adnate.

only did suppression occur: in S<sub>2</sub>/Br<sub>2</sub>/2 the right antero-lateral member was entirely absent.

D. *Posterior staminode*. The fifth stamen, in the form of a staminode, was normally present. Some doubt arises as to whether this structure is generally present in this species. It is figured by Le Maout and Descaigne (8) as a minute tongue of tissue, and Braun (3), in reference to *Antirrhinum*, speaks of the rudimentary stamen: Chevannes (5) also notes its occurrence. Penzig (12) leads one to suppose that the occurrence of the staminode is an abnormality. Hooker, in the 'Student's Flora,' states that the fifth stamen is 'rudimentary or  $\sigma$ ' in the genus. It may well be that the staminode is of normal occurrence, but sometimes overlooked on account of its minuteness, or this stamen may at times be present and at others missing.

In B/1 it was certainly absent, but in B/2 it was a large hooked structure (Fig. 6 A), with a groove running along it; at its extreme tip it was slightly petaloid; it was adnate to the corolla for about a fifth of its length. In B/3 the staminode was probably present as a small flattened structure fused by its edges to the corolla, but since in this flower the corolla was opened for examination by a posterior incision it was difficult to obtain exact information from this region. In B/4 it consisted of a minute tongue-like structure with two points, but again its precise nature was difficult to elucidate, as the corolla was opened posteriorly. B/5, 7, and 8 all had a minute staminode similar to those of the S series described below. This structure appeared to arise a short distance above the base of the corolla, but this probably represented the free tip of the staminode, the remainder being adnate to the corolla. B/6 had a large staminode (Fig. 9 F) similar to that of B/1.

In all the Slough flowers which were examined the staminode was present. In what is regarded as the more or less normal condition, as seen in most of the flowers, it consisted of a minute tongue of tissue, practically as broad as long, and fused to the corolla for about half its length (Fig. 9 A). Filamentous processes were not infrequently present in connexion with this stamen in the S flowers, but no instance was noted in which paired processes occurred. The filamentous processes arose either on the right or left side of the staminode, and may reasonably be looked upon as appendages, equivalent to those on the functional stamens, since they occur with less regularity than the tongue-like process which has been termed the staminode, and since, moreover, the vascular strand runs up this process and not up the lateral appendages. The appendages varied from short teeth (Fig. 9 B, C) to filiform bodies many times longer than the staminode itself (Fig. 9 D, E): usually the basal part of the appendage was adnate to the corolla, and sometimes not more than half of it was free.

E. *Androeceum: Summary and theoretical considerations*. The occurrence of abnormalities in the androeceum of *Antirrhinum majus* has been

noted by a number of botanists. I have not been able to trace records of fertile stamens with petaloid tips such as those figured in Fig. 8 I, but Chavannes (5) has figured similar stamens for *Linaria vulgaris*, 'dont les

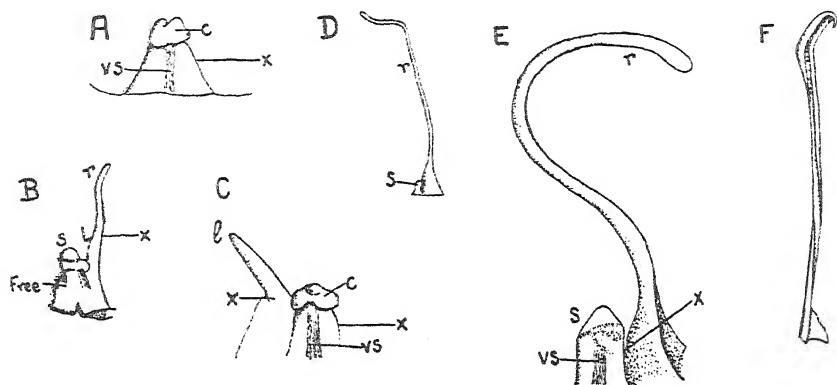


FIG. 9. Staminodes selected from various flowers. All  $\times c. 6$ , except D and F, which are  $\times 2$ . A, S1/3, typical staminode of S flowers. B, S1/5. C, S3/6. D, S1/2. E, S3/7, staminodes bearing appendages. In E the staminode is attached to the corolla only at its base. F, B/6, an unusual type of staminode. c, cap. l, left-hand staminodal appendage. r, right-hand staminodal appendage. s, staminode. vs, vascular strand. x, indicates point above which staminode or its appendage became free.

connectifs des étamines sont métamorphosés en lames petaloïdes'; he also records a stamen entirely petaloid.

Two types of filiform or petaloid bodies are recorded: (1) those replacing stamens, (2) those developed in addition to stamens. Morren (11) examined flowers of a sterile variety of *Antirrhinum* known as 'Mufle de veau blanc double' and found that the stamens were replaced by hollow tubes (ascidia) ending in a petaloid flap, sometimes reflexed. These structures agree with those described in this paper, save that in the former the limbs were hollow tubes and not rolled bodies produced from a flat limb. Morren noted the appearance of the posterior stamen at times as a white filament as long as the tubular stamens: it may be noted that his figure of the normal flower shows no trace of the staminode. Masters (10) records the presence of 'two small green laminae on the outer side of the two posterior stamens', and regards them as probable developments of the thalamus—'a kind of foliaceous disc'. Braun (3) found in some flowers from two to six thread-like structures developed in close relation to the stamens; these occurred on one side or the other of the stamen, but not on both sides, but the very small staminode had one on either side; he regarded these threads as bodies of a stipular nature. Eichler (6) noted the frequent occurrence, in the androeceum of cultivated plants, of staminode-like leaflets on either side of the rudimentary fifth stamen; these were sometimes present on the fertile stamens, and in their most highly developed form were narrow, petaloid leaflets; on the normal



stamens they were sometimes only small, filiform structures; he never found them to possess anthers, and noted their more frequent occurrence on abortive stamens. He was inclined to regard them as subsidiary leaf formations (stipules) of the filaments.

The appendages of the flowers of the B and S series appear to be of the same nature as those described by Braun and Eichler. It is of interest, however, that they occurred in these plants less frequently on the staminode—where they were never paired—than on the fertile stamens, and were no less common on the fertile stamens than on the abortive ones. Their fairly constant occurrence, position, and mode of origin, suggest that they are stipular bodies, but this view is advanced with reserve, since stipules are of rare occurrence on the leaves of members of the Tubiflorae: the occurrence of stipules in normally exstipulate groups is, however, not unknown; Arber (1, 2) records their presence in a very rudimentary or, at least, undeveloped form in a number of Cruciferous plants, and it seems customary to regard the nature of the paired outgrowths of the filaments in certain species of *Allium* as stipular.

Bureau (4) has described flowers of *A. majus* in which there were appendages, which he interpreted, after examination of the course of the vascular strands, as appendages of the corolla comparable to the scales on the petals in some of the Caryophyllaceae; in the absence of figures it is not possible to ascertain how nearly these structures correspond to the staminal appendages, but one inclines to the view that they were possibly staminal appendages partially adnate to the corolla. Bureau found that the structures were supplied with vascular strands from the corolla, but this does not necessarily militate against the view that they are staminal appendages. Although the vascular strands of the flowers described in this paper have not been studied in great detail, vascular strands have been traced from the corolla to the appendages; these might well be branches of the main vascular strand to the stamen, given off at the base of the corolla.

Certain stamens of the B flowers (Fig. 8 K, L) have been interpreted above as undeveloped stamens bearing well-developed appendages. In connexion with this interpretation it is worthy of note that Arber (2) finds paired squamules or stipular bodies in certain Crucifers, in association with the pedicels, and suggests that they 'represent the stipules of the absent bract'.

#### 4. *Abnormalities in the Gynaecium.*

*A. Trilocular ovaries.* Several ovaries in both the B and S flowers were trilocular. All such ovaries were sectioned and examined with care. Two possible explanations may be put forward to account for the phenomenon. (1) That a third carpel has appeared. (2) That the condition originates by a splitting of the anterior placenta, and the subsequent

appearance of a septum between the two halves. It may be pointed out in favour of the former view that the capsule of *Antirrhinum* dehisces by three pores, two communicating with the anterior loculus and one with the posterior loculus, and it is possible that the two anterior pores originally communicated with separate loculi. From an examination of herbarium material of *Antirrhinum* at Kew it would appear that the posterior pore is not of quite the same nature as the anterior ones. It is not infrequently elongated transversely, and appears sometimes to open as two pores which ultimately communicate by a transverse slit. In *A. sempervirens* Lap. a somewhat similar condition was noted in the anterior loculus of one or two fruits, so it appears that the two anterior pores communicate at times, if rarely.

In support of the alternative view is the fact that tricarpeal ovaries are of rare occurrence in the whole of the Tubiflorae, although the formation of secondary septa is common enough in such families as the Boraginaceae, Labiatae, and Verbenaceae, while it occurs in isolated examples in the Solanaceae, e.g. *Datura*.

On the whole, the author inclines to the view that the trilocular condition arose by the formation of a secondary septum, and the description, which follows, of these ovaries, illustrated in Fig. 10, is based on this view. Fig. A shows a fairly normal condition, in which the anterior loculus is somewhat larger than the posterior one. Fig. B again illustrates the bilocular condition, but here the anterior placenta is concave along its free face; ovules occurred along the concave region. In the same ovary a section near the base showed that the concavity was more extensive and devoid of ovules; each end of the original placenta now formed a placenta, separated from its fellow by a septum running from the anterior wall of the ovary to the centre of the concave region of the placenta, and thus producing two loculi in place of the single original anterior one. In this section the posterior loculus was cut through at its extreme base, and the regions *p.p.* merely represent depressions in its floor. The secondary septum sometimes formed practically the whole of the wall separating the two daughter placentas (Fig. E), but sometimes part of the wall was formed by the stalk of the original placenta, i.e. the split affected the placenta only and not its stalk; in such cases the daughter placentas appear to be not strictly axile (Fig. D). The septum was thinner than the ovary walls or the original septum separating the posterior and anterior loculi, and, as is seen in Figs. C and D, did not always pursue a straight course; it was sometimes somewhat wrinkled. It did not necessarily meet the divided placenta in the centre (Fig. F). It was usually noted that the division of the anterior loculus affected the anterior wall of the ovary, which became concave (Figs. D and F) like the lateral walls of the normal ovary.

Chavannes (5) figures an ovary of *Antirrhinum majus* which is trilocular,

and which he states is formed from three carpels. The figure appears to represent a normal tricarpellary ovary in which each loculus, and all three septa, are equally developed.<sup>1</sup>

B. *Incomplete enclosure of placentas.* Where the loculus of an ovary

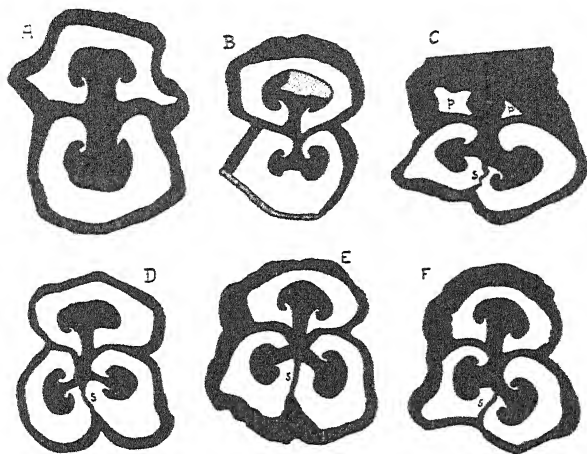


FIG. 10. Camera lucida drawings of transverse sections of ovaries: ovules omitted. In all cases the posterior margin of the ovary is at the top.  $\times 8$ . A,  $S_3/5$ . B,  $S_2/B_{r1}/4$ , near top of ovary. C,  $S_2/B_{r1}/4$ , near base of ovary. D,  $S_2/B_{r1}/3$ . E,  $S_2/B_{r1}/1$ . F,  $S_2/6$ . *p*, extreme base of posterior loculus. *s*, presumed secondary septum. Shaded parts were missing from the section.

was in communication with the exterior, the style and stigma were abnormal. These anomalies were not frequent, and are of sufficient interest to merit a detailed description.

In B/1 the style was a short stout structure with a hooked tip (Fig. 11, B). On the concavity of the hooked tip there was an opening, covered by a flap, which led, via a hollow, somewhat membranous tube, into the anterior loculus of the ovary. In B/2 the gynaecium was similar, but with a less curved tip (Fig. 11, A); the mouth of the tubular part of the style was at the tip, *a*, and was not closed by a flap. It is doubtful if a stigma existed in either flower, or if pollination was possible.

The gynaecium of B/3 was a most unusual structure. The posterior part consisted of a much reduced placenta bearing ovules (Fig. 11, J, *p.p.*), and five leaf-like structures, F and F', *a-e*, of which two were posterior, and fused by their edges near the base, I; these two segments apparently formed the wall of the posterior part of the ovary, J, *ba*, and *bb*. Of the remaining leafy members, which were more anteriorly placed, two were fused at the base in the middle of their broad faces, and the third,

<sup>1</sup> I have found trilocular and quadrilocular ovaries in *Lindenbergia grandiflora* (Ham), but these have not yet been fully investigated.

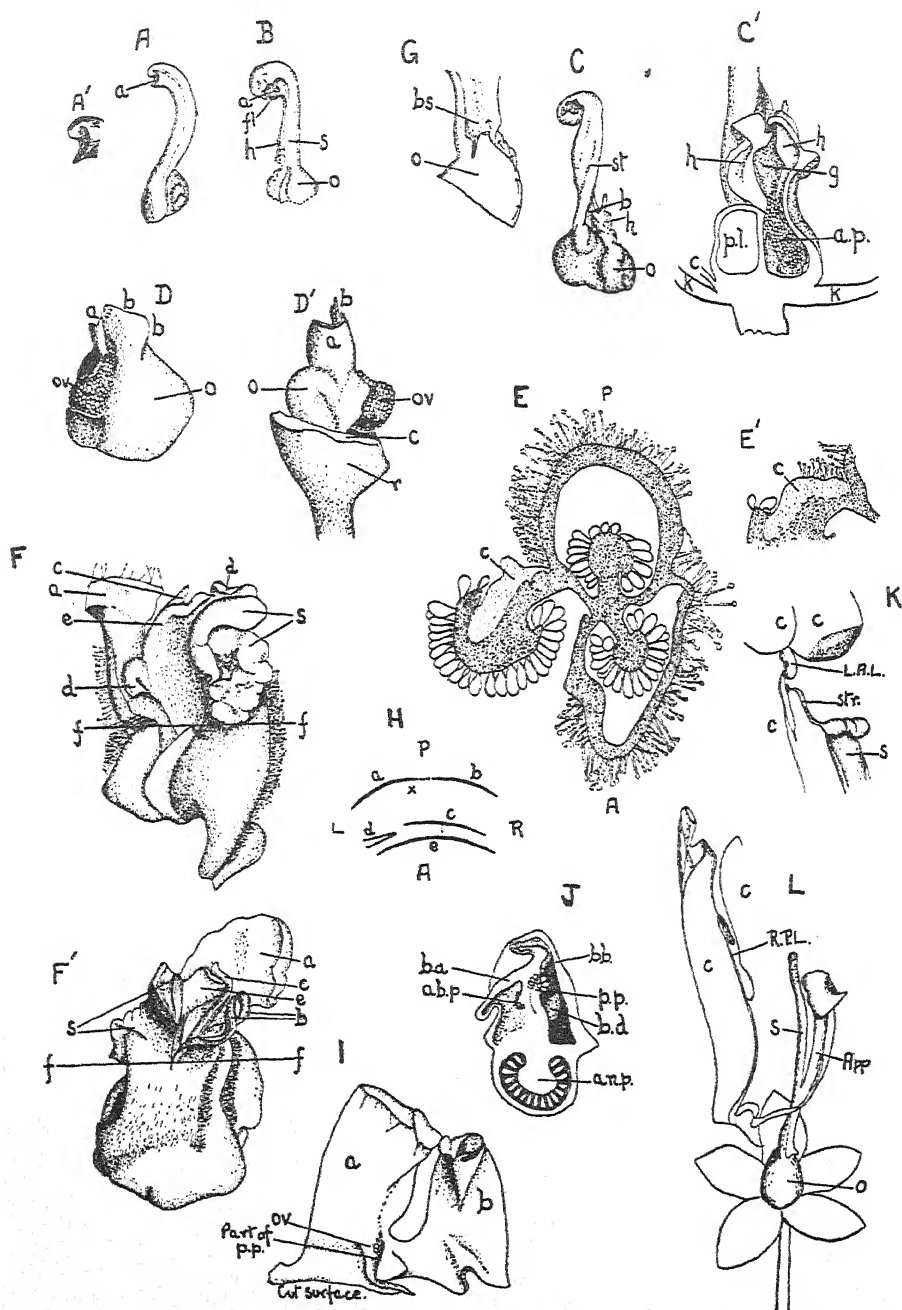


FIG. 11. Abnormalities in gynaecium. A-C'. Incomplete enclosure of anterior placenta. A, B/2, style and stigma and top of ovary: shows anterior tubular region of style.  $\times 2$ . A', B/2, top of style, showing opening of tubular region.  $\times 4$ . B, B/1, gynaecium: top of ovary only is

lying on the left-hand side and forming a little tongue-like structure, F, *d*, was folded on itself along its long axis. There is little doubt that the placenta arose from the margin of the left posterior leafy segment. The anterior loculus had a normal placenta, J, *an. p.*, but along the anterior margin there was free communication to the exterior at the base of the style, via a somewhat membranous tube, G, and F, *s.* The style itself was a flattened gutter-like structure, of no great length; from its distal end there arose a somewhat ellipsoidal, curved structure, and a much convoluted membranous body, which was in communication with the membranous tube along the anterior edge of the loculus.

In S<sub>2</sub>/Br<sub>2</sub>/2 the style and stigma was a flattened gutter-like structure, bent over distally, C, *st.* Along the anterior edge of the ovary was a membranous tube, which opened at one end into the anterior loculus and at the other into the base of the style.

S<sub>2</sub>/5 presented probably the most extraordinary anomaly encountered. It bore a short flattened apical region, D and D', *a, b*, which probably represented the style and stigma. The ovary was more or less normal, except that on the posterior loculus, on the left side, there occurred a patch of naked ovules, D and D', *ov.*: sections of the ovary showed two normal placentas in the usual position, while there was a third arising from the outer side of the wall of the posterior loculus, E. The external placenta was of the usual shape, but rather larger than the two normal ones; posteriorly it possessed a patch of tissue composed of rather larger cells than the remainder, E, *c.* This tissue, it was thought, might conceivably have formed a sort of core to the placenta, the posterior part of this structure having been torn in sectioning. However, the adjacent section showed that

shown. × 2. C, S<sub>2</sub>/Br<sub>2</sub>/2, gynaecium from left. × 2. C', nearly median *l.s.* gynaecium, showing incomplete enclosure of anterior placenta. The groove, *g*, opens at the base of the style (at *b*, in Fig. 11 C). Posterior placenta omitted. × c. 3. *a.*, distal end of tubular part of style. *a.p.*, anterior placenta. *b.*, base of style. *c.*, corolla. *f.*, flap covering opening in style. *h.*, tubular region of style. *k.*, calyx. *o.*, ovary. *p.l.*, posterior loculus. *s.*, style. *st.*, flattened style. D-E', S<sub>2</sub>/5, a gynaecium with an external placenta. D, gynaecium from left. × c. 3. D', gynaecium from posterior side. × c. 3. E, *l.s.*, ovary. (Camera lucida drawing, slightly diagrammatic.) × c. 13. E', part of adjacent section to E, showing patch of large-celled tissue, bearing hairs. × c. 13. *a* and *b*, style and stigma? *c*, cut base of calyx. *d*, patch of tissue with cells rather larger than those of the rest of the walls and placentas. *o.*, ovary. *ov.*, ovules on external placenta. *r.*, receptacle. P and A, mark posterior and anterior positions respectively. F-J, B/3. F, gynaecium from left. × c. 7. F', gynaecium from right. × c. 7. G, anterior part of gynaecium, showing base of style and top of ovary. Arrows mark passage of bristle from loculus to exterior. × 7. H, diagram of arrangement of leaf-like structures connected with posterior loculus. I, anterior side of the two posterior leaf-like structures, showing ovules. × c. 7. J, *l.s.*, ovary, semi-diagrammatic. × c. 7. *a, b, c, d, e*, leaf-like structures connected with posterior loculus. *ab.p.*, abortive placenta? *an.p.*, anterior placenta. *ba.*, base of *a. bb.*, base of *b. bd.*, part of *d. bs.*, opening at base of style. *f.f.*, line marking approximate point where the leaf-like members become free. *ov.*, ovules on *a. pp.*, posterior placenta. *s.*, abortive style and stigma. *x.*, marks position of posterior loculus. P and A, mark posterior and anterior positions respectively. R and L, mark right and left hand sides respectively. K, S<sub>2</sub>/Br<sub>2</sub>/1, showing attachment of top of style and stigma to corolla. × 2. *c.*, corolla. L.A.L., left antero-lateral anther. *s.*, top of style and stigma. *str.*, strand from top of style to corolla. L, B/4, showing attachment of style to right postero-lateral stamen (R.P.L.). × 2. *app.*, appendage of R.P.L.? *c.*, corolla. *o.*, ovary. *s.*, style.

hairs occurred on the superficial cells in this region,  $E'$ ,  $c$ , which suggests that the tissue was external.

These abnormalities are difficult to interpret. Ovaries opening to the exterior are of normal occurrence in *Reseda*, and as abnormalities are not infrequent in other plants. It is not easy to understand why the connexion always occurs through a styler tube occupying an anterior position, although it may possibly be accounted for on the grounds that there is more room for the development of the anterior side of the style than the posterior side. The peculiar gynaecea of  $B/3$  and  $S_2/5$  are regarded as monstrous, and it is not without significance to note that they occur in distinctly abnormal flowers. A possible explanation of the external placenta of  $S_2/5$  is that it represents an appendage, comparable to a staminal appendage, and arising from the posterior carpel: it is a serious objection, however, that in no instance were fertile staminal appendages noted, nor have they been recorded by other authors. In the same way one might regard the leafy members of  $B/3$  as the tops of the carpellary leaves and their appendages; in this case,  $a$  (Fig. 11 H) would be the posterior carpel, with a single appendage,  $b$ ;  $c$  would be the top of the anterior carpel,  $d$  and  $e$  its appendages.

*C. Cohesion.* Cohesion occasionally occurred between the gynaeceum and other whorls. In  $B/4$  what was probably the petaloid appendage of the right postero-lateral stamen was attached for about half its length to the style (Fig. 11, L). The distal part of the appendage was free, and from this region arose a strip of tissue, joined at its other end to the base of the right postero-lateral stamen. It is perhaps possible to explain the structure as due to the fusion of part of the appendage to the base of the style, the rest remaining attached to the stamen; by unequal growth of gynaeceum and corolla the base of the appendage may have become split, forming thus the strip of tissue described.

The apex of the flattened style of  $S_2/Br_2/1$  (Fig. 11, K) gave rise to a thin strand of tissue, *str.*, which terminated in a small boss fused to the filament of the left antero-lateral stamen, just below the anther. The filament of the stamen was broken, but it could not be ascertained whether the break was due to insect attack or to irregularities of growth.

### 5. On the Nature of Abnormalities.

The plants described above show so many digressions from the normal that they provide material for a consideration of the nature of abnormalities. The validity of treating abnormalities as reversionary has recently been questioned by Arber (2); while the present writer is not in agreement with all the views expressed in her paper, the subject has, nevertheless, been so adequately summarized that it would be superfluous to deal with it here. The question here considered is one raised by Masters (9); how far are

abnormalities to be regarded as monstrous, that is, as malformations produced by some derangement of the normal metabolism of the species, and thus pathological. The alternatives are that they are atavistic, or natural, representing extremes of variation.

A normal structure is one which appears in a large number of individuals of a species under consideration: it will show considerable fluctuation about a mean. The extremes of such variation will occur but seldom, and may be met so rarely as to be described as abnormalities when they are found. Examination of a sufficiently large number of individuals might place the so-called anomaly in its proper place; but there is no reason to assume that the extremes will always be found, however diligently they may be sought; they may be of such rarity as only to occur at intervals, although still connected with the normal by a range of minute gradations. It is not unreasonable to expect an individual plant with the potentiality for producing these extremes to produce them more than once, for example, in several flowers, but not everywhere as would happen were the organism a mutant. Were such a feature to occur but once, without connexion to the normal by a series of intermediates, there would be justification for regarding it as a malformation.

Where the anomaly is such that the organ in which it occurs is incapable of fulfilling its normal function, one is justified, in general, in interpreting it as a monstrosity. Caution is necessary; it would be unreasonable to consider a normally occurring staminode as a malformation on the grounds that it is a non-functional stamen: the same applies to such structures as the stamens of the Marantaceae and Cannaceae. That malformations can occur is evident when the abnormalities produced by parasites are considered. The parasite, by upsetting the normal metabolism of its host, may bring about hypertrophy. Unknown factors, producing abnormalities in metabolism might bring about a similar result. There is, of course, no reason why the metabolism should not vary continuously about a mean, and its extremes be responsible for extreme morphological variations.

Further, it should be possible to interpret a natural anomaly in terms of the normal, of the species, or at least of a related organism, and as the normal can often be interpreted in terms of phylogeny, so also should it be possible to interpret the anomaly. For example, a normal Angiospermic carpel can be regarded as a folded megasporophyll, bearing marginal ovules in its basal region: a carpel in which style and stigma are replaced by a leaf-like structure will bear the same explanation. On the other hand, a carpel bearing anthers externally, as described by Salisbury (14), does not lend itself to a similar interpretation; there is no evidence of bisporangiate sporophylls in the Phanerogams; such a structure, in the opinion of the writer, is a monstrosity, arising perhaps by cohesion between members of two distinct whorls, or possibly produced spontaneously.

Considering the anomalies in the *Antirrhinum* flowers, the following are regarded as representing rarely encountered extremes of normal structures:

- a.* Fission of the corolla.
  - b.* Enation of the stamens: these showed, in the same inflorescence, every gradation between the extreme type with two petaloid appendages and the stamen normally encountered in the species.
  - c.* Variation in the staminode.
  - d.* Perhaps petaloidy of the stamens, at least in those cases where there were normal anthers.
  - e.* Trilocular ovaries.
  - f.* Perhaps incomplete enclosure of placentas.
- The following are considered to be malformations:
- a.* Synanthly of the flower.
  - b.* Alteration in plane of symmetry of the flower.
  - c.* Sterility of the stamens.
  - d.* Gynaecea of B/3 and S<sub>2</sub>/5.
  - e.* Cohesion of parts of gynaeceum to other whorls.

It seems to be justifiable to regard these features merely as malformations when one considers their very sporadic occurrence, and the fact that, in general, they are not connected by intermediates to the normal structures. Further, in many cases they occur in flowers showing several anomalies, and such abnormalities, in the developing bud, may well have affected the meristems producing the different whorls, and thus have led to the production of monstrous structures.

#### SUMMARY.

A number of anomalies in flowers of *Antirrhinum majus* L. are described and discussed. Of these the more outstanding are:

1. *Synanthly*: in which two flowers formed a single bud.
2. *Variation in the plane of symmetry*: a flower in which the wall between the two loculi of the ovary was oblique.
3. *Enation*: stamens frequently carried paired appendages, which are compared with stipules.
4. *Sterility and Petaloidy of stamens*: including stamens with well-developed appendages, but with the fertile segment undeveloped.
5. *Occurrence of Posterior Staminode*: in varying form, sometimes with an appendage.
6. *Trilocular Ovaries*: the origin of which is traced from the normal bilocular type.
7. Other abnormalities in the ovary, including *incomplete enclosure of*



*loculi*, a *naked placenta*, and the presence of *leaf-like structures*. The nature of abnormalities in general is discussed.

What remains of the flowers on which this investigation was made has been deposited at the Herbarium of the Royal Botanic Gardens, Kew.

Thanks are due to Prof. T. G. Hill and Prof. E. J. Salisbury for advice and criticism, to Miss R. E. Dowling, who furnished me with the Slough material, and to Mr. J. Ramsbottom for facilities to study certain plants in the British Museum Herbarium. For the microscopical work, use was made of the microscope lent to me by the Dixon Fund for other researches.

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#### *Postscript.*

Owing to the uncertainty which appears to exist regarding the staminode of *A. majus*, publication of this paper was postponed until a large number of flowers could be examined. Through the kindness of Messrs. Sutton and Sons, Ltd., I was able to examine some hundreds of flowers of numerous varieties of this species, and also of some hybrid

experimental forms. I have also examined flowers of mixed Intermediate varieties raised from seed obtained from Messrs. Toogood and Sons, Ltd., and from Messrs. Alexander and Brown, and also some of unknown origin. With the exception of two flowers noted below, and of eight flowers of an inflorescence of a hybrid, no. 66,<sup>1</sup> a staminode was always present. It varied somewhat in form. In general it was under 5 mm. long, often minute and scarcely visible to the unaided eye, although easily seen with a lens. In a few cases it was as much as 10 mm. long, and in some of the hybrid flowers occasionally nearly 20 mm. It was invariably free from the corolla, except near the base. Sometimes the staminode was filamentous, but not infrequently the tip was somewhat forked or tufted, this being produced, apparently, by abortive anthers. In a few cases the tips bore structures which were undoubtedly poorly developed anthers, and all stages between this and the filamentous type of staminode was observed.

Examination of so large a number of flowers brought to light a number of anomalies. Most were of the type recorded above, but one or two were not noted in flowers of the B or S series. In one variety there were many anomalies in the flowers; one flower of this variety, occurring near the base of a raceme, was peloric and tetramerous, showing four sepals, four petals each with a saccate base and with the free tips reflexed, and four stamens. Clefts in the corolla were of not infrequent occurrence, and in a few flowers these occurred between the two postero-lateral petals, separating them nearly or quite to the base. Stamens were noted bearing appendages similar to those already described, and in a few cases filaments were adnate to the corolla; petaloid flaps above the anthers were occasionally present. A more unusual abnormality occurred in two flowers. In one there were three stamens on either side, all of which, except the middle one on the left side, had anthers, although these were poorly developed; there was no staminode in this flower. In another flower there was a stamen between the left postero-lateral one and the staminode. This stamen had a short filament with an apparently abortive anther, and bore on its left side a large hooded appendage adnate to the stamen.

Several anomalies were noted in the staminode. Occasionally this bore an appendage on either side; these appendages were sometimes as long as the stamens and of the hooded, petaloid type, and these were sometimes associated with a staminode under 1 mm. long. The appendages were sometimes long and filamentous, sometimes minute. In a few flowers two minute staminodes occurred side by side, quite free from one another and with separate vascular strands. In one flower these two staminodes were fused together, but showed independent vascular strands. In one

<sup>1</sup> Plants referred to by a number are varieties of Sutton's Little Gem, a group produced by crossing Sutton's bedding strain of *Antirrhinum majus* with *A. glutinosum*; the numbers are those used at Messrs. Sutton's trial grounds to distinguish the plants.

flower there was no staminode, although on either side of the point from which the staminode normally arises there was a filamentous structure, suggesting appendages of an undeveloped staminode.

Numerous anomalous ovaries were found. One flower, of an unknown variety, had the septum between the two loculi almost in the antero-posterior plane, while in no. 57 a flower was found in which the posterior loculus was better developed than the anterior one, a condition never again encountered. The most interesting anomalies were, however, encountered in a number of Messrs. Sutton's experimental types, including some of the hybrid forms. Four main types were noted. (A) The normal bilocular type. (B) A unilocular ovary with a roughly mushroom-shaped placenta arising from one or other lateral wall. (C) An ovary with free central placentation. (D) Sterile ovaries with a single loculus, or sometimes an ovary consisting of a solid mass of tissue. All four types were often encountered on the same raceme, and in such cases, in general, the normal ones and type B were about equally frequent, while the C and D types were perhaps a little less common.

A possible explanation of the B type is that one margin of each carpellary leaf, and consequently the ovules on that side, failed to develop, so that the ovary was formed by the apposition of the two leaves, fused by their edges on one side and inrolled in the usual manner on the other. If this be so, it might be expected that the number of vascular strands in the wall of the B type should be smaller than that in the normal type. As will be seen from Table II this is so. It is not possible, however, to account for the free central type of ovary in this manner, and, as the table shows, this has fewer vascular strands than either types A or B. Fewer vascular strands still, occurred in the sterile ovaries.

TABLE II.

*Approximate Number of Peripheral Vascular Strands in Different Types of Ovary.*

	Normal.	Type B.	Type C.	Type D.
No. 60 . . . . .	43, 52, 54	39, 42, 49	30, 41	25, 33
Buff Pink . . . . .	57	45, 49, 49, 50, 54	—	—
Tom Thumb, Tall Apricot . . . . .	55	43	35	—
Bedding Yellow . . . . .	58, 64, 68, 69, 80	45, 47	30, 43, 51	30

It is suggested tentatively that the type B arises through the suppression on one side or the other of the septum separating the two loculi. Some support for this view is found in ovaries which are of the B type above, but normal nearer the base. The C type may be accounted for by the suppression of the septum on both sides, and here it was not uncommon

to find an ovary which appeared free central above but of the B type lower down. The sterile type may arise from the free central type by the gradual decrease in size of the placenta, and its final disappearance. It seems not unlikely that these anomalies were due to irregularities in nutrition.

In two flowers the ovary was of the B type, but the placenta arose from the posterior wall of the ovary. This type of ovary is probably not related to the B type, but arises simply by suppression of the posterior locus.

It is intended to make the anomalous ovaries the subject of a more extended investigation as soon as possible.

I desire to thank Mr. A. P. Balfour and Dr. W. B. Turrill for assistance in connexion with the work mentioned in this postscript.

# The Effect of Temperature on the Geotropism of Seedlings of *Lathyrus odoratus*.

BY

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With four Figures in the Text.

## I. INTRODUCTION.

DURING the course of some earlier work on the geotropism of seedlings (9), it was noted that the effect of change of temperature on the length of presentation time and latent time was very great. Bach (2), Rutgers (15), Maillefer (12) and others have published the results of experiments at different temperatures, but these authors did not take into account the normal changes in sensitivity to gravity during the development of a seedling (9). The present work is, therefore, an attempt to give a somewhat fuller account of the effects of temperature on the geotropism of seedlings and to investigate the manner in which a change of temperature is able to influence geotropic sensitivity.

*Lathyrus odoratus* was selected for detailed study, since the results of experiments on seedlings of this species have already been published (9) and it has been found to provide convenient material. In the previous experiments with *L. odoratus*, seeds of mixed origin were used, but it was thought that more exact results might be obtained if a named variety were used. Accordingly experiments were performed at 20° C. with the following named varieties, *Majestic Cream*, *Constance Hinton*, *George Shawyer*, *Warrior*, *Black Knight*, *Dorothy Eckford*, *Mrs. Tom Jones*, *Royal Purple*, and *Grenadier*. No significant difference in presentation time or latent time could be detected in these widely differing varieties, and hence mixed seeds were again used in the present work.

## 2. EXPERIMENTAL METHODS.

The seeds were sown in small wooden boxes of a uniform size containing a mixture of approximately equal parts of sand, sifted loam, and sifted leaf-mould, and were germinated and grown in an electrically

controlled germinator at a temperature of  $20^{\circ} \pm 5^{\circ}$  C., unless otherwise stated.

The seedlings were transferred to the experimental temperature for one hour before the experiments were commenced. Rutgers (15) also exposed his material to the experimental temperature for one hour before stimulation and found that this period was both necessary and sufficient. Experiments were performed at temperatures of  $5^{\circ}$  C.,  $10^{\circ}$  C.,  $15^{\circ}$  C.,  $20^{\circ}$  C.,  $25^{\circ}$  C.,  $30^{\circ}$  C.,  $35^{\circ}$  C., and  $40^{\circ}$  C. Experiments at temperatures of  $20^{\circ}$  C. and over, were performed in a cubical box (side 1 foot), fitted with a sliding glass lid and suspended in a water bath, the temperature of which was controlled by a mercury thermo-regulator. Experiments at lower temperatures were performed in a similar sized box around which ice was packed in sufficient quantity to produce the required temperature. With this apparatus it was found to be possible to obtain extremely constant temperatures and, when there was any deviation from the required temperature, no experiments were performed. All experiments were performed in the dark.

Presentation time and latent time were found by the same method as that previously described (9).<sup>1</sup>

### 3. EXPERIMENTS AT DIFFERENT TEMPERATURES.

Detailed results have been obtained for geotropic presentation time at arbitrarily chosen stages in the growth of the seedlings, and these results are given in Table I and Figs. 1 and 2. Values for latent time have not been calculated for each stage in the development of the seedling, but are given for the period of maximum sensitivity (i.e. for epicotyls of 6–10 cm. in length) only. Presentation time has been worked out in greater detail than latent time, since the former gives a measure of *sensitivity* to gravity while the latter merely indicates the power of response of the stimulated organ, with which the present paper is not primarily concerned.

The length of the geotropic presentation time is seen to decrease with increase of temperature between  $5^{\circ}$  C. and  $30^{\circ}$  C., reaching a minimum value at  $30^{\circ}$  C. and then rising rapidly with further increase of temperature. Latent time shows a similar minimum value at  $30^{\circ}$  C. Thus the sensitivity to gravity of epicotyls of *L. odoratus* increases with increase of temperature up to  $30^{\circ}$  C., after which the seedlings rapidly become less sensitive. Similar results were obtained over a range of temperature between  $10^{\circ}$  C. and  $25^{\circ}$  C. with epicotyls of *Asparagus officinalis*.

The results obtained for presentation time are in general agreement

<sup>1</sup> Presentation Time was taken to be the period of stimulation necessary to cause response in 75 per cent. of the seedlings used. Latent Time was taken to be the period from the beginning of stimulation to the point at which 75 per cent. of the seedlings showed a visible curvature.

TABLE I.

*Geotropic Presentation Time of Epicotyls of Lathyrus odoratus at Different Temperatures.*

Length of epicotyls in cm.	Presentation time in minutes.							
	5° C.	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.	40° C.
0-1	80	45	35	20	16	15	17	35
1-2	70	35	25	16	12	11	13	25
2-4	65	30	20	12	8	7	9	20
4-6	62	27	17	9	5	4	6	16
6-10	60	26	16	8	4	3	5	15
10+	65	32	20	12	8	7	9	20
Latent time at period of maximum sensitivity	180	100	55	35	31	30	32	50

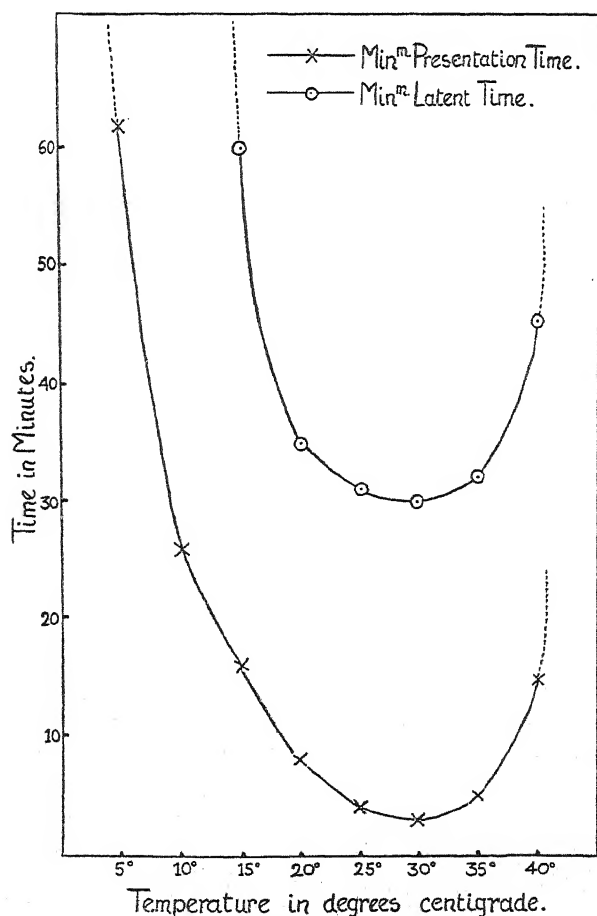


FIG. 1. Graph to show relationship between temperature and geotropic presentation time and latent time at the stage of maximum sensitivity of the epicotyl of *Lathyrus odoratus*.

with those of Bach (2) for epicotyls of *Vicia Faba* and those of Rutgers (15) for coleoptiles of *Avena sativa*, but do not entirely agree with Czapek's (6) results for primary roots of *Lupinus albus*, since Czapek found no change in presentation time between 15° C. and 30° C.

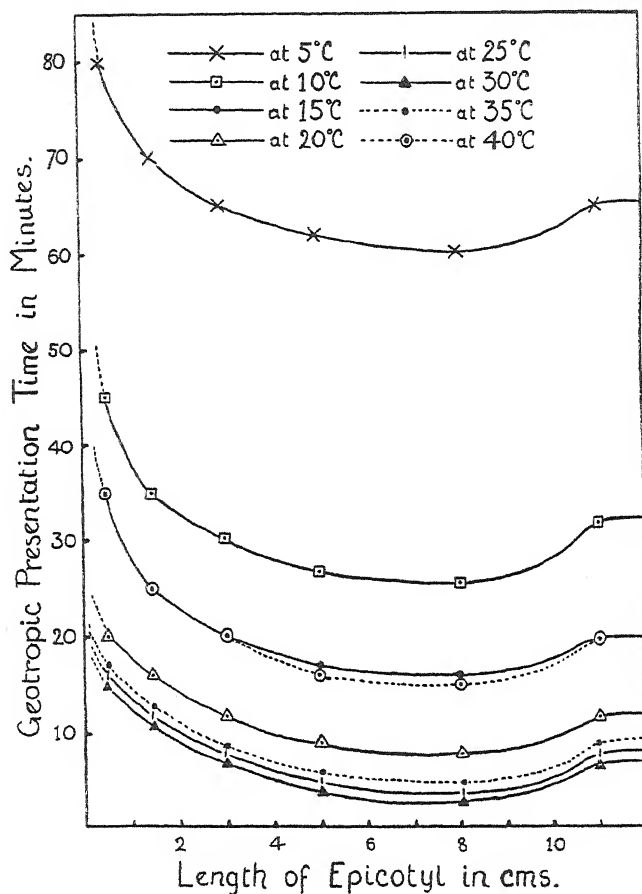


FIG. 2. Graph to show effect of temperature on geotropic presentation time during the development of the epicotyl of *L. odoratus*.

The results obtained for latent time are also in general agreement with those of Bach, but Rutgers considered that latent time (or reaction time) was not dependent on temperature, a result which was probably due to the fact that he did not leave his seedlings at the experimental temperature during response.

It is of interest to trace the correlation between changes induced by temperature in the functioning of the statolith apparatus of the epicotyl and the changes in presentation time described above. Even in the case of the lower temperatures, the time during which the seedlings were



exposed to the experimental temperature was too short to make any measurable alteration in the amount of statolith starch present. Measurements were made, by a method previously described (9), of the rate of fall of the statoliths at different temperatures. The results obtained are given in Table II, the *minimum* geotropic presentation times (i.e. the presentation times at the stage of maximum sensitivity) for each temperature being given for comparison.

TABLE II.

*Rate of Fall of Statoliths in Epicotyl of L. odoratus at Different Temperatures.*

Temperature.	Rate of fall of statoliths in $\mu$ per hour.	Minimum presentation time.
10° C.	26	26
20° C.	54	8
30° C.	106	3
40° C.	38.5	15

The rate of fall of the statoliths, being dependent on the viscosity of the protoplasm in the statocyte, increases with increase of temperature up to 30° C. and then falls off rapidly, thus showing a close correlation with changes in sensitivity to gravity.

These results agree with those of Weber (16) who reported a decrease in the time of fall of statolith starch grains in seedlings of *Phaseolus multiflorus* with increase in temperature. Weber, however, does not give any corresponding figures for geotropic presentation time. The present results also agree generally with those of Pantin (14) on changes in the viscosity of the protoplasm of *Nereis* eggs with change in temperature, but are contrary to the majority of the results published on the effect of temperature on the viscosity of protoplasm. Heilbrunn (11), using *Cumingia* eggs, reported a *maximum* viscosity at 15° C. and Heilbronn (10) obtained a similar maximum at 21° C. with *Myxomycetes*. Baas Becking, Bakhuyzen, and Hotelling (1) report a maximum viscosity at 27° C. with cells of *Spirogyra*. These conflicting results are difficult to explain, but it is of interest that all measurements made on changes in viscosity with temperature, as indicated by the rate of fall of statoliths are in agreement.

It is notable that the temperature coefficient of rate of fall of statoliths is 2, which is higher than that usually obtained for physical processes. Hence it is indicated that rate of fall is governed by some other factor than the viscosity of protoplasm alone.

It thus seems probable that part, at any rate, of the increase in

geotropic sensitivity with increase of temperature up to 30° C. can be accounted for by increased rate of fall of the statoliths.

#### 4. EXPERIMENTS ON THE PREVIOUS TREATMENT EFFECTS OF TEMPERATURE.

Bach (2) has reported that periods at a low temperature reduce the sensitivity to gravity of seedlings of *Vicia Faba* even when they are restored to a higher temperature. He does not, however, give any precise data on this subject, and the periods during which his material was in the cold varied from 5½ to 28 hours. It was thought, therefore, that a series of more critical experiments on the previous treatment effects of temperature would be likely to yield results of interest, and accordingly such a series of experiments was devised and carried out.

A. Seeds of *L. odoratus* were germinated at 20° C., in order to avoid introducing the additional factor of a long period of germination, and were then placed in a germinator kept at a temperature between 5° C. and 10° C. When the seedlings had attained a suitable size, experiments were performed at 20° C. and 30° C.

It was found to be difficult to compare the results obtained in this series with the results, described above, with seedlings grown at 20° C., since the epicotyls become more elongated when grown at the higher temperature. An attempt was made to use the number of nodes unfolded as a criterion of 'stage of development' instead of length, but this was abandoned since the nodes are too far apart to give a sufficiently close series of stages and are also very irregularly spaced. Finally it was decided to determine the period of maximum sensitivity in each case, and to compare the presentation time at this period (i.e. the minimum presentation time) in each batch of seedlings.

It has already been found that this minimum presentation time for seedlings grown at 20° C. and stimulated at 20° C. and 30° C. is 8 and 3 minutes respectively (see Table I). The minimum values of presentation time at 20° C. and 30° C. with seedlings grown at 5° C. were found to be 25 and 11 minutes respectively, while the corresponding latent times were increased by growth at 5° C. from 35 and 30 minutes to 60 and 45 minutes respectively. Thus growth at 5° C. decreases the geotropic sensitivity of the epicotyl to about one-third of the sensitivity of epicotyls grown at 20° C.

B. Seedlings of *L. odoratus* were grown at 20° C. and transferred to a temperature of 5° C. 24 hours before stimulation. They were then transferred to the experimental temperature (20° C. or 30° C.) for one hour before stimulation. After 24 hours at 5° C. the minimum presentation time was found to be 8 minutes at 30° C. and 16 minutes at 20° C. Latent time showed a similar increase. Thus a period of 24 hours in the cold

reduces the sensitivity of the epicotyl, but to a smaller extent than a long period in the cold, the sensitivity in the former case being reduced to about half that of seedlings grown at 20° C.

C. Further experiments were carried out to see if restoration to a temperature of 20° C. could bring about partial or complete recovery from the effects of 24 hours at 5° C. Accordingly, seedlings of *L. odoratus* were grown at 20° C. and then exposed to a temperature of 5° C. for 24 hours, as in Series B, but were restored to a temperature of 20° C. for a further period of 24 hours before stimulation. The presentation times found for seedlings, so treated, at 20° C. and 30° C. were found to be 12 and 5 minutes respectively, indicating that partial recovery had taken place.

D. A further series of experiments was carried out in which seedlings which had been exposed to a temperature of 5° C. for 24 hours were restored to a temperature of 20° C. for 48 hours before stimulation. Recovery was then found to be complete. The results of experiments A, B, C, and D are set out in Table III.

TABLE III.

Series.	Minimum <sup>1</sup> presentation time at 30° C.	Minimum presentation time at 20° C.	Minimum latent time at 30° C.	Minimum latent time at 20° C.
A. germinated at 20° C., grown at 5° C. . . . .	11	25	45	60
B. germinated and grown at 20° C., exposed to 5° C. for 24 hrs., then stimulated . . . . .	8	16	—	—
C. as in B., but restored to 20° C. 24 hrs. before stimulation . . .	5	12	—	—
D. as in B., but restored to 20° C. 48 hrs. before stimulation . . .	3	8	30	35
E. germinated and grown at 20° C. (Data taken from Table I for comparison). . . . .	3	8	30	35

An attempt was then made to investigate the way in which previous treatment at a low temperature is able to reduce the sensitivity of seedlings when restored to a higher temperature. Haberlandt (8) has reported the complete disappearance of statolith starch in plants grown at 0° C. It therefore seemed probable that the amount of statolith starch in the epicotyl of *L. odoratus* would be reduced by exposure to a temperature of 5° C. even though the fact that response can still take place at 5° C. suggests that statolith starch does not disappear altogether at this temperature. Accordingly the volume of statenchyma (or statolith-containing cells) present in the epicotyls of seedlings grown at 5° C. was measured by

<sup>1</sup> i.e. at the period of maximum sensitivity.

the method used in earlier work (9). The average amount found was 0.675 c.mm.,<sup>1</sup> which is only about one-fifth of the amount previously found to be present at the stage of maximum sensitivity in seedlings grown

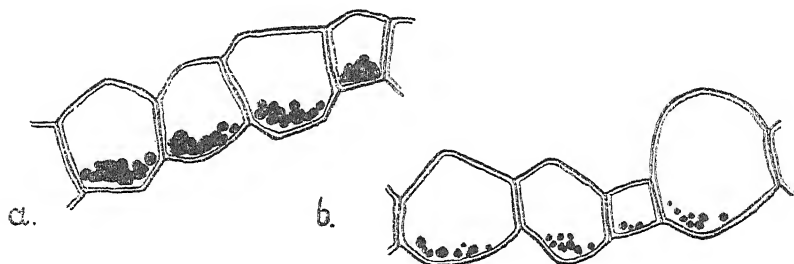


FIG. 3. (a) cells, containing statoliths, from the endodermis of the epicotyl of *L. odoratus* grown at 20° C.  $\times 360$ . (b) cells, containing statoliths, from the epicotyl of *L. odoratus* grown at 5–10° C.  $\times 360$ .

at 20° C. This amount is even less than would be expected from a consideration of the values of presentation time, but this discrepancy can be accounted for by the fact that the significant difference previously calculated (9) for results obtained in this way is 0.755 c.mm. There was also considerably less starch in each statocyte in the case of those seedlings grown at 5° C. than in those grown at 20° C. (Fig. 3).

In the case of seedlings in series B and C, which had only been exposed to the low temperature for 24 hours, no decrease in the amount of statolith starch in the individual statocyte nor in the total volume of statenchyma in the epicotyl could be detected. The rate of fall of the statoliths was also found to be the same in series B and C, as that found for epicotyls of seedlings which had been grown entirely at 20° C. Accordingly it seemed likely that the decrease in sensitivity caused by 24 hours' exposure to a temperature of 5° C. must be due to the influence of this treatment on some other part of the complicated process of geotropic perception and response. It has been established by Cholodny (4), Went (17), and others that geotropic curvatures are caused by a re-distribution of some growth-regulating substance or hormone, within the stimulated organ. It seems probable that the effect of a short exposure to the cold is to check the rate of production of the growth-substance, by the re-distribution of which curvature is supposed to result. In order to test this assumption, seeds of *Vicia Faba* were germinated in damp sawdust at 20° C. Half of the seedlings were transferred to a temperature of 5° C. for 24 hours and the remainder were left at a temperature of 20° C. After this period of 24 hours the roots were decapitated and the following four

<sup>1</sup> These figures refer to actual volume and not to relative volume, since no significant difference was found between the area of the cross-section of the epicotyl in the two batches of seedlings.

series of experiments were set up in a damp chamber at a temperature of 10–15° C. (the four series are shown graphically in Fig. 4).

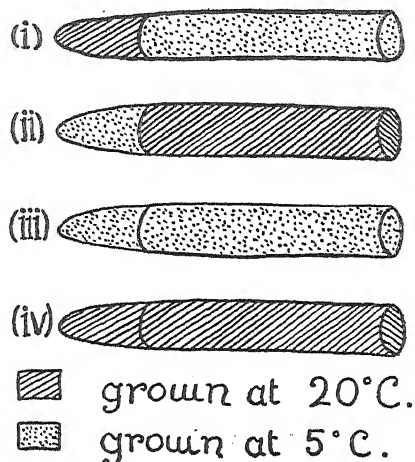


FIG. 4. Explanation in text.

- (i) Tips from seedlings grown entirely at 20° C. were stuck on to the decapitated stumps of seedlings which had been exposed to the low temperature.
- (ii) Conversely tips from roots which had been exposed to the low temperature were stuck on to the stumps of roots which had been left at 20° C.
- (iii) Roots which had been exposed to the low temperature were re-headed with their own tips.
- (iv) Roots which had been left at 20° C. were re-headed with their own tips.

These roots were then placed in a horizontal position in the damp chamber. After 24 hours the following results, set out in Table IV, were obtained.

TABLE IV.

Series.	No. of seedlings used.	Number responding.	Average curvature.
(i)	15	13 (86·7 %)	30·8
(ii)	9	1 (11·1 %)	5
(iii)	13	5 (39 %)	18
(iv)	10	9 (90 %)	28·8

The results of this experiment indicate that a short period in the cold is able to decrease the sensitivity of the root of *V. Faba* by decreasing either the amount or the rate of production of growth-hormones in the tip. It seems highly probable that decrease in the amount of hormone present

is also responsible for the similar decrease in sensitivity in the case of the epicotyl of *L. odoratus*, but it is more difficult to demonstrate this fact in the case of the epicotyl, owing to the absence of conductance of the 'stimulus' over any considerable distance.

The probable significance of these results will be discussed later.

## 5. DISCUSSION OF RESULTS.

From the values of the presentation time (given in Table I), for the period of maximum sensitivity (i.e. in seedlings with epicotyls of 6–10 cm. in length), the following temperature coefficients can be obtained:—

	Q 10
5° C.–15° C.,	60/16 = 3.7
10° C.–20° C.,	26/8 = 3.25
15° C.–25° C.,	16/4 = 4
20° C.–30° C.,	8/3 = 2.7

Thus the average temperature coefficient between 5° C. and 25° C. is 3.75, which is considerably higher than the values obtained by most other observers. Rutgers (15), using coleoptiles of *A. sativa*, obtained a temperature coefficient of 2.6 for a range of temperature between 5° C. and 30° C. He shows how, by taking the time factor into consideration and obtaining, by calculation, a theoretical value for presentation time, after zero time at the experimental temperature, van't Hoff's law could be made applicable to his results; this method having been used by Blackman (8) in studying the effect of temperature on photosynthesis. Czapek's (6) figures give a temperature coefficient of 2.25 between 5° C. and 15° C. The results of Navez (13) and Maillefer (12) are chiefly concerned with 'reaction time'. Bach (2), however, obtains figures for presentation time, from which a temperature coefficient of 3.75 can be calculated for a range of temperature between 20° C. and 30° C., thus agreeing more closely with the results of the present writer.

If, however, from the results given in Table I, the *average* presentation time be calculated for each temperature (i.e. if changes in sensitivity during the development of the seedling be ignored) a temperature coefficient much more nearly approaching that of Rutgers is obtained. The average presentation times at temperatures of 5, 10, 15, 20, 25, 30° C. are 47, 32.5, 22.2, 12.8, 8.5, 7.8 minutes respectively, giving the following temperature coefficients:—

	Q 10
5° C.–15° C.,	2.1
10° C.–20° C.,	2.5
15° C.–25° C.,	2.6
20° C.–30° C.,	1.9

Since apparently neither Rutgers nor Czapek took the age of the seedlings used into consideration, it appears likely that the apparent discrepancy between the temperature coefficients calculated from their results and from the present series of results can be explained in this way, while the higher temperature coefficient obtained by Bach may be due to the fact that he used seedlings of some particular age, although he does not actually state this.

The remarkable fact thus arises from the results given in Table I, that temperature has actually a greater effect on the geotropic sensitivity of *Lathyrus* epicotyls of 6–10 cm. in length (i.e. at their period of maximum sensitivity) than on younger seedlings and also has a slightly lesser effect on the sensitivity of epicotyls over 10 cm. in length. It thus seems probable that limiting factors may govern sensitivity of gravity in much the same way as was suggested by Blackman (3) in the case of photosynthesis. In the case of geotropism the factors which 'limit' sensitivity are probably internal factors, i.e. the rate of re-distribution of growth-hormones (shown to precede curvature in the stimulated organ by Cholodny (5), Gradmann (7), and others) and the efficiency of the statolith apparatus. It is conceivable that both the fall of the statoliths and the re-distribution of hormones are necessary stages in the chain of events between perception and response. If this be the case, then the poor development of the statolith apparatus may be a 'limiting factor' in very young seedlings (9), while in seedlings of maximum sensitivity the statolith apparatus has become so well developed that the rate of re-distribution of hormones in the stimulated epicotyl is now the limiting factor. In the case of slightly older seedlings the reduction of the statolith apparatus in the epicotyl, which has previously been described (9), may be sufficient to limit sensitivity once more. Such a hypothesis can account for the changes in  $Q_{10}$  value during the development of the seedling and also for the high  $Q_{10}$  obtained at the stage of maximum sensitivity, since the temperature effect is here a combination of the effect of temperature on the rate of fall of the statoliths and on the re-distribution of the hormones.

From the results of the experiment described above, (Table IV and Fig. 4) in which tips from roots which had been chilled for 24 hours were stuck on the stumps of roots which had remained at 20° C., and vice versa, further deductions can be drawn, and it appears that a short period in the cold is able to influence geotropic sensitivity by its influence on hormone production. Diffusion of hormone takes place at the same rate in the previously cooled roots and in the uncooled roots, since series (i) and (iv) in this experiment give similar results. The effect of cooling on viscosity does not, therefore, remain after re-warming, and hence previous cooling does not reduce sensitivity and response through a reduction in the rate of conduction of the stimulus. Since it has already been shown that a short

period in the cold does not reduce the efficiency of the statolith apparatus when the seedlings are restored to a temperature of 20° C., it seems probable that it either (a) reduces the supply of some substance essential to hormone production, or (b) directly reduces the supply of the hormone itself. This supposition is supported by the fact that, if an uncooled root tip (which would, presumably, contain the normal amount of hormone), be stuck on to a previously cooled root stump, the response of the latter is greatly increased, and, indeed, is nearly as great as the response of a root which has never been cooled.

From these observations, it seems highly probable that the fall of the statoliths is a necessary stage preceding that re-distribution of growth-hormones which ultimately causes response. Much evidence has also been collected by numerous investigators to show that both the statolith apparatus and the presence of growth-regulators are essential to geotropic response. It thus becomes conceivable that the fall of the statoliths in some way actually leads to the re-distribution of growth-hormones within the stimulated organ.

Zaepfell (18) has pointed out that, in the presence of diastase the heaping up of the statolith starch grains on the lower wall of the statocyte (or statolith-containing cell) would cause a greater concentration of sugar on the *upper* side of that wall than on the lower and would thus lead to a flow of water from the lower to the upper cell. He supports this suggestion by experiments with an artificial membrane, through which he obtained such a flow of water.

It does not seem altogether improbable that such an alteration in the water relations of the statocytes might ultimately lead to a re-distribution of the growth-hormone in the stimulated organ, although the method by which this re-distribution takes place is not yet clear.

## 5. SUMMARY OF RESULTS.

1. Geotropic Presentation Time and Latent Time for the epicotyl of *Lathyrus odoratus* decrease with increase in temperature up to 30° C., after which they increase rapidly.

2. Temperature has a greater effect on presentation time in epicotyls of maximum sensitivity (i.e. 6–10 cm. long) than in younger epicotyls or in slightly older epicotyls.

3. The rate of fall of statolith starch increases up to 30° C. and then rapidly decreases, showing a close correlation with sensitivity to gravity.

4. Growth at a low temperature (i.e. 5° C.) reduces sensitivity to gravity even when the seedlings are restored to a temperature of 20° C. or 30° C. The statolith apparatus is also reduced by growth in the cold.

5. 24 hours in the cold reduces sensitivity to gravity even when the



seedlings are restored to a temperature of 20° C. for 24 hours before stimulation, but the effects of the 24 hours cooling do not remain after 48 hours at the higher temperature. Evidence is given that the effect of 24 hours cooling is to reduce the supply of growth-hormone.

6. It is suggested that the fall of the statoliths in some way brings about the re-distribution of growth-hormones in the stimulated organ, which has been shown to precede curvature.

My sincere thanks are due to Dr. T. A. Bennet-Clark for many helpful suggestions made during the course of this work and to Professor M. Drummond for kindly reading the manuscript and making suggestions for its improvement.

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# The Monoblepharidales.<sup>1</sup>

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With Plate XX and two Figures in the Text.

## INTRODUCTION.

AMONG the Phycomycetes no other group presents such unusual features as the small and somewhat isolated order of the Monoblepharidales. Discovered over sixty years ago in France by Cornu, it remains unique to-day, not only from the standpoint of the seemingly infrequent occurrence of its members, but also by reason of the fact, that it is, thus far, the only group of fungi in which a relatively large, non-ciliated, and practically motionless egg is fertilized by an active and disproportionately small, ciliated sperm. In these morphological characters it approaches not only certain groups of algae but some of the more primitive types of animals as well.

In the present paper an account of the morphology and development of *Monoblepharis* (and to a lesser degree of *Gonapodya*), and a taxonomic treatment of the order are given.

## HISTORICAL ACCOUNT OF ORDER.

In 1871, Cornu (5) published his discovery of a new genus of aquatic fungi which, in contrast to all other known forms, possessed motile sperms. Three species, *Monoblepharis* (*Gonapodya*) *prolifera*, *M. sphaerica*, and *M. polymorpha*, were briefly characterized at that time. The following year, in his classic 'Monographie des Saprolegniées' (6), the last two fungi were more completely described and illustrated, and a brief description, without figures, of the first, *M. prolifera*, was included. The latter species was figured two years later in the 1874 edition of Van Tieghem's 'Traité de Botanique' <sup>3</sup> (French edition of Sachs' 'Lehrbuch').

<sup>1</sup> Contribution from the Cryptogamic Laboratories of Harvard University, no. 113.

<sup>2</sup> National Research, Fellow in the Biological Sciences.

<sup>3</sup> See p. 537.

Twenty-five years elapsed before any members of the genus were again observed. Indeed, as Thaxter (21), presumably the second person to encounter these organisms, suggested, mycologists were doubtful whether or not a group of fungi with such unique characters actually existed. With the appearance of Thaxter's paper in 1895, all doubts were dispersed, and our knowledge of the genus *Monoblepharis* was considerably enhanced. Further investigations, especially those of Lagerheim (12) in Sweden and Woronin (26) in Finland, have greatly extended our knowledge, as has the more recent work in Germany by Laibach (13) on the cytological aspects of the group.

#### METHODS OF COLLECTION.

##### *Monoblepharis.*

It has been the writer's experience that most members of the genus may be obtained from a single favourable situation. Further, the supposed rarity of these forms appears largely due to the lack of information concerning proper methods of collection and subsequent treatment of material.

Species of *Monoblepharis* are primarily inhabitants of dead, entirely submerged twigs in permanent, fresh-water habitats. In the collection of such twigs, several factors should be borne in mind. First, the pool must be relatively quiet and free from silt in suspension and from products of organic decomposition. Secondly, the twigs collected must not be decorticated and should preferably be waterlogged. Thirdly, certain types of twigs, notably those of birch and ash, seem particularly favourable for the requirements of the fungus.

Other types of substrata, such as animal cadavers (insects?), twigs of other broad-leaved trees, needles, twigs, and sap of coniferous trees, submerged lichens and fungi, and fruits have also been described as favourable for the development of *Monoblepharis*.

Material of the fungus seems rarely to be found on twigs brought in from the field and immediately subjected to examination. If, however, such twigs are placed in sterile, distilled water, and maintained at 8–15° C. for three to seven days, the fungus, if it is present on the substratum, will by then have produced an abundance of growth. In such cases there will appear tufts or pustules of very delicate, pale grey, rather flexuous hyphae, which may cover the twig or be confined to the openings of the lenticels. If, subsequently, material thus obtained is maintained at 8–11° C. only sporangia will develop, whereas, if the same culture is placed at room temperature (21° C.) sexual reproduction will occur.

*Gonapodya*.

Species of *Gonapodya* are frequently found occurring on twigs with *Monoblepharis*. However, they are much more common on submerged fruits, particularly those of apple and rose. On these substrata the fungus will form a loose, filmy mass, or, more frequently, it will occur in definite pustules on the surface of the fruit in association with *Blastocladia* and *Rhipidium*. The surest method of obtaining such fruit-inhabiting fungi is to construct traps of galvanized wire screening, place fruits in them and submerge them in some likely aquatic habitat. The fruit in such traps when left for at least a month will usually yield an abundance of material of *Gonapodya* and other Phycomycetes. After examination, such fruits may be placed in jars with a relatively large amount of water, left at a low temperature (3–8° C.) and examined at intervals. Further details of these culture methods may be found in the papers of Kanouse (9, 10).

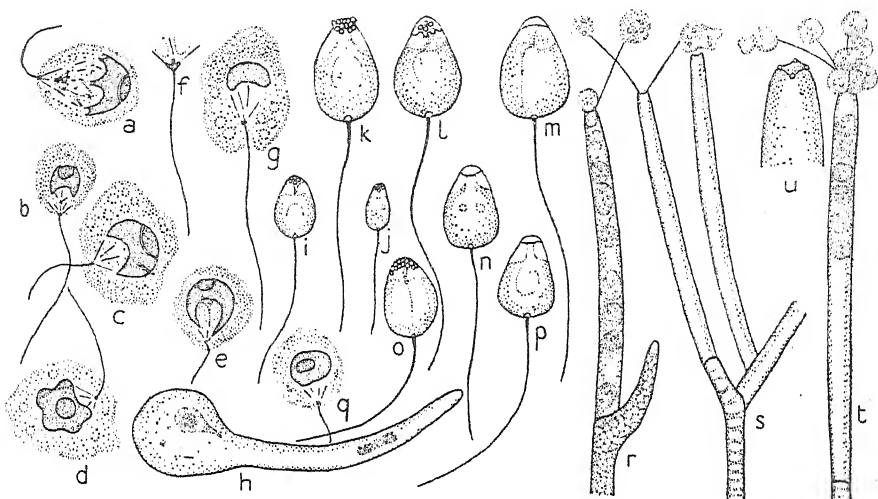
DEVELOPMENT AND LIFE HISTORY OF *MONOBLEPHARIS*.

The zoospore upon germination may produce two germ-tubes, one of which gives rise to the rhizoidal system which anchors the plant to the substratum, the other producing the main body of the plant. According to Lagerheim (loc. cit.) rhizoids may also be formed by the ramifying hyphae.

*Mycelium*. Once the fungus is established there results, under favourable conditions, an abundant mycelial growth, the nature and extent of which appears to depend somewhat on the particular species. For example, in *M. macrandra* the growth and branching may be exceedingly profuse and may result in a solid mat of interlocking, tangled hyphae covering the substratum, whereas in the other species the filaments seem more rigid, less branched, and tend to remain separated from one another. The finely granular content of the hyphae, in which are occasional refractive fat granules, is usually disposed, due to the regularly placed vacuoles, in a reticulate or foamy manner. The striking effect produced by this type of vacuolization makes it comparatively easy for one to recognize, even in the vegetative condition, a member of the *Monoblepharidales* (Pl. XX, Figs. 7, 32). Under changing environmental conditions the protoplasm may temporarily assume a non-vacuolate, homogeneous texture.

*Sporangia*. At about 8–11° C. only non-sexual reproductive organs are formed. These are usually terminal, slightly swollen, cylindrical portions of the hyphae which possess homogeneous contents. Each sporangium is finally separated by a cross wall from its adjacent hypha. According to Laibach (loc. cit.), the nuclei are at first more or less regularly placed in the hyphae. As the sporangium 'Anlage' begins to form there is an increase in the number of nuclei in that body. No mitotic figures were observed in the

process of sporangial formation by either Lagerheim (loc. cit.) or Laibach. It remains a question, therefore, whether the increase in the number of nuclei is due to a migration of the latter bodies into the sporangium or to



TEXT-FIG. 1. Non-sexual reproduction of *Monoblepharis* and *Gonapodya*; a-g, *M. polymorpha*, cytological preparations of the zoospores; f, greatly enlarged drawing of point of attachment of cilium showing rod-like character; all other figures  $\times 1260$ . h, cytological preparation of germinating zoospore showing what is apparently a stage in the division of the nucleus in the germ-tube,  $\times 1260$ . i, moving zoospore of same species,  $\times 675$ . j, antherozoid of *M. polymorpha* drawn to same scale as i. k-m, views of various zoospores of same species, in motion (freehand). n-q, *G. prolifera*. n-p, zoospores in motion,  $\times 675$ . q, cytological preparation of zoospore,  $\times 1200$ . r, discharging sporangium of *M. polymorpha*,  $\times 375$ . s, cluster of sporangia of *M. macrandra*,  $\times 375$ . t, sporangium of same species with a cluster of emerging zoospores at its apex,  $\times 375$ . u, rectangular orifice with four glistening nodules, sometimes observed in sporangia of this species (freehand). To prevent confusion, the cilia of the zoospores of these figures are not drawn to their full length.

the division of a few nuclei. As the cross wall is laid down, the nuclei become more or less equidistant from one another. A central vacuole is present as differentiation of the zoospores is initiated. Coincident with spore cleavage there is a marked increase in the size of the individual nucleus, which now exhibits around its periphery masses of dark-staining material. Laibach suggests that the latter material may be concerned with cilia formation.

In sporangia observed by the writer, the cleavage planes of the spores appear in most instances to be at right angles to the long axis of the sporangium, although many may also be obliquely placed. The spore initials are at first angular in outline, but gradually become more rounded. During this process of cleavage all of the protoplasm may not be used up in the formation of the spores, and small bits may remain in the sporangium after discharge. By the deliquescence of the tapering apex of the sporangium a circular, sometimes angular (Text-fig. 1, u) pore is formed, through which

the swarmers creep in an amoeboid fashion to the outside medium (Text-fig. 1, *r, s, t*). Outside, the zoospore remains adherent by its cilium to the mouth of the sporangium for a varying length of time. Here it may oscillate for some little time before finally becoming disengaged (Text-fig. 1, *s*). In the meantime other spores may continue to emerge, and it is not uncommon to see three or more spores thus attached to the orifice of the sporangium (Text-fig. 1, *t*). The zoospore at this time exhibits an almost homogeneous content in which are imbedded a few refractive granules. When the spore, by a quick succession of vigorous jerks, ultimately becomes entirely free from the sporangium, it either immediately darts away or floats for a time feebly lashing its cilium. The latter, which in length is about four to five times the diameter of the spore body, slowly increases its rate of vibration and ultimately, trailing behind, propels the spore in a smooth, gliding, lively fashion through the water. After complete discharge of the zoospores further sporangia may be formed by cymose branching of the hypha (Text-fig. 1, *s*) or, in *M. regignens* and occasionally in *M. sphaerica* and *M. ovigera* (Pl. XX, Fig. 23), by proliferation through the empty sporangium.

If a recently discharged zoospore be followed in its course, a remarkable transformation in its internal structure will be observed. The latter, at first nearly homogeneous, assumes a definite, characteristic internal arrangement which enables one to recognize free swimming spores of this genus as well as those of *Gonapodya* (Text-fig. 1, *i, n, o, p*). When the spore is in motion, the aforementioned refractive granules come to occupy the most distal portion of the now more cylindrical spore body. Indeed, these granules often appear to protrude from the somewhat acuminate, slightly quivering apex (Text-fig. 1, *k*). Sometimes these refractive bodies seem to be fused into a single, broadly cone-like structure (Text-fig. 1, *l, m, n, p*). Immediately beneath there is a space entirely devoid of granular material. A narrow band or strand connects the granules with the rest of the spore body or possibly with the nucleus. The greater part of the spore is of a very finely granular, slightly refractive protoplasm which appears in some views to be a band, the two ends of which have fused (Text-fig. 1, *m*). At the point of insertion of the cilium a highly refractive body may usually be found. The little-known figures of zoospores and antherozoids given by Cornu in Van Tieghem's 'Traité de Botanique' indicate that he was well aware of this characteristic internal structure.

A preliminary cytological examination of the spores, using, with slight modifications, the method outlined by Cotner (8), reveals the following facts.

The nucleus has a general resemblance in its top-like or sub-triangular shape (Text-fig. 1, *c*) to that found in *Blastocladia* (8) and more particularly in *Allomyces* (11). Frequently the broader end terminates in a darker

staining lenticular structure (Text-fig. 1, *a-e*), whereas the opposite, concave edge may often extend in strands towards the point of insertion of the cilium (Text-fig. 1, *a, e*). At the latter point there is a definite dark-staining body separated from the broad end of the nucleus by a space which, while relatively free from stain, contains a series of radiating 'fibrils' (Text-fig. 1, *a-e*). This basal granule, which may be regarded as the blepharoplast, often appeared to consist of radiating rod-like structures (Text-fig. 1, *f*). In only two instances was the blepharoplast found in a peripheral position on the spore (Text-fig. 1, *a, b*) after the latter was prepared for cytological examination, although it seemed to occupy this position in the motile swarmer.

The protoplasm of the anterior portion of the spore is much more foamy in appearance than the remainder. Spores which when killed were obviously moving in an amoeboid manner seem to be rather uniformly vacuolate (Text-fig. 1, *d, g*). The anterior refractive granules noted in the living spore were not stained by gentian violet. Upon germination, the nucleus of the zoospore divides, and further divisions take place as the hypha grows in length (Text-fig. 1, *h*). More precise and exhaustive work on this phase of the life history of *Monoblepharis* should yield abundant results.

*Sexual reproduction.* Details of the sexual reproduction of *Monoblepharis* were accurately portrayed by Cornu, and the observations of the few subsequent workers have added little to it, except cytological details. However, as these organisms have been seen so infrequently, it has seemed justifiable to include here the development of the sex organs and details of the process of fertilization as they occurred in the present material. It is worthy of note that no significant points of difference were exhibited by the various species in the act of fertilization.

In *M. polymorpha*, *M. fasciculata*, and *M. insignis* the antheridia appear at first glance to be inserted on the oogonia (epigynous). A study of their development, however, indicates that the oogonium is formed beneath the antheridium and is an intercalary structure. In such a typical epigynous form as *M. polymorpha* the antheridium originates as a walled-off terminal portion of the hypha, the contents of which are rather homogeneous (Pl. XX, Fig. 8). The more proximal portion of the hypha just beneath the antheridial cross wall then gradually becomes more distended and forms a lateral, somewhat oblique projection (Pl. XX, Fig. 9). This lateral distention continues (Pl. XX, Fig. 10), and a clavate body eventually separated by a basal cross-wall from the hypha is formed (Pl. XX, Fig. 11). Often, before the formation of the septa, the antheridium has discharged its antherozoids (Pl. XX, Fig. 11). The oogonium thus delimited gradually becomes more rotund in shape.

Maturation and escape of the antherozoids are accomplished in the



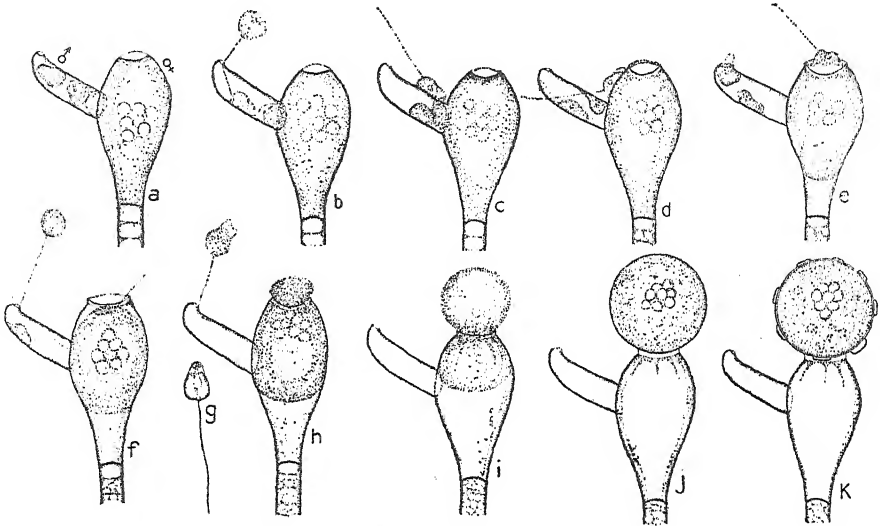
same manner as was described for the zoospores (Text-fig. 2, *a, b, c*). The antheridia, however, in contrast to sporangia, bear only relatively few motile bodies (4–8). In their shape, internal structure, and ciliation, these resemble zoospores (Text-fig. 1, *j*; Text-fig. 2, *g*). Unlike the latter, however, they are smaller and exhibit a pronounced tendency for amoeboid movement.

Further maturation of the oogonium involves the formation of a highly refractive, apical, receptive papilla (Text-fig. 2, *a*). Coincident with, or often before the formation of this structure, the minute, evenly disposed oil droplets in the ooplasm combine to form a number of large, refractive globules. These, as the maturing of the egg progresses, may come to occupy a central position in the oogonium. Both Lagerheim and Laibach have shown that the egg is uninucleate, and that the nucleus ultimately attains a terminal position in the oogonium. This nucleus may be easily demonstrated by mounting material *in toto* in weak cotton-blue and lactophenol (Pl. XX, Fig. 7).

Apparently fertilization is possible only after the egg has reached the proper stage of maturity. It was frequently observed that antherozoids creeping over immature eggs could not fertilize them. Often, too, they seemed unable to fertilize ova which, by their appearance, were fully mature. Whether or not in the last instance this was due to the fact that these eggs were only seemingly mature, or that antherozoids from androgynous antheridia were unable to self-fertilize their own oosphere is in need of further investigation. The latter explanation does not seem to suffice in all instances, however, for numerous examples of self-fertilization were witnessed. In some cases the amoeboid motion of the antherozoid seemed markedly accelerated upon coming into contact with the oogonium. On the other hand, free swimming antherozoids would often pass in close proximity to unfertilized, mature eggs without being visibly attracted to the latter. If the oogonium does possess any chemically attractive property for the antherozoids, it is either not diffused to any great distance out into the water or is attractive to only certain ones. In one instance a sperm was observed struggling violently to get free from the receptive papilla. Immediately after freeing itself, the oogonial wall dilated and the ooplasm emerged. If this egg had previously been fertilized, as seems likely, the chemotactic response of the struggling antherozoid, after coming into contact with the papilla, seemed quite a negative one. Further, the inability of the sperm to free itself at once would seem to give support to Cornu's suggestion that some sort of mucus retains it on the papilla.

When an antherozoid approaches the apex of the oogonium (Text-fig. 2, *b, c, d*) the peripheral collar of the wall of the latter, until then contiguous with the papilla, dilates slightly (Text-fig. 2, *d*). The sperm resting on the papilla (Text-fig. 2, *e*), which seems to be an integral part of

the ooplasm, is immediately engulfed (Text-fig. 2, *f*), and both sperm and papilla become relatively indistinguishable from the egg. The cilium of the sperm may protrude for a few moments, but it, too, is finally absorbed.



TEXT-FIG. 2. Sexual reproduction of *M. polymorpha* (all  $\times 675$ ). *a, b*, emergence of antherozoid from antheridium; *c, d*, antherozoid creeping toward receptive spot of oogonium; the oogonial wall around the receptive spot has begun to dilate; *e, f*, antherozoid being engulfed by the ooplasm; after this, the ooplasm retreats into the oogonium; *g*, antherozoid in motion, note the difference in internal structure from that of amoeboid one; *h-j*, emergence of oosphere, the protoplasm of antherozoid may still be distinguished; *k*, early stage in formation of bullate wall of oospore (combined views).

After fertilization the egg, which has previously become more compact and has moved towards the apex of oogonium, retreats slightly and remains motionless for a few minutes. After three to five minutes it expands, and there is initiated a gradual evacuation from the oogonium (Text-fig. 2, *h, i*). The time required for this process varies, but it usually takes at least two minutes. The wall of the oogonium is again dilated as the egress of the egg commences. In rare instances the protoplasm of the antherozoid may still be distinguished after discharge of the egg (Text-fig. 2, *i, j*).

Outside, the egg remains attached to the mouth of the oogonium by a narrow, hyaline collar. A pellicle soon forms around the egg, which gradually thickens, and on which there appear regularly placed protuberances (Text-fig. 2, *j, k*). The latter increase in size and become the bullations so characteristic of the oospore of most species of the genus. Woronin (loc. cit.) found the oospore to be composed of two main layers, exospore and endospore. The exospore was, in turn, of two parts. Of these, the inner, in *M. sphaerica*, was thick and colourless and was raised to form the bullations; the outer portion was thin, brown, and did not cover

the warts. In *M. macrandra*, however, the outer, brown layer covered the bullations. Within the exospore the living protoplasm was surrounded by a thin, nearly colourless, elastic wall (endospore).

As Lagerheim, and later Laibach, observed, the male and female nuclei in the egg do not at once fuse, but remain side by side until wall formation has reached an advanced stage and the bullations are beginning to form. Fusion then takes place, and the mature oospore is uninucleate.

Only a few cases of germination of the oospore have been observed by the writer. In several oospores which were estimated to be not more than a month old, the wall of the spore had cracked open and a single hyphal-tube had been produced (Pl. XX, Fig. 39). Under the existing conditions only mycelium was formed. No other type of germination was seen either by Lagerheim or Laibach. According to the latter, in *M. macrandra*, the large resting nucleus of the oospore may divide into as many as sixteen perceptibly smaller nuclei. No mitotic figures were observed, but it was supposed that reduction took place during the first division. Upon the formation of the germ-tube the nuclei migrate into the latter structure, and the vegetative mycelium is established. Further cytological work is greatly needed on this and other critical points in the life history, particularly on those of an epigynous species.

*Monoblepharis sphaerica* and *M. macrandra*, while possessing essentially the same types of sex organs as those of *M. polymorpha*, differ from the latter species in their methods of development.

In *M. sphaerica*, the oogonial 'Anlage' is formed first in a terminal position (Pl. XX, Fig. 2). After the oogonium is delimited another more proximal hyphal segment is blocked off, which becomes the hypogenous antheridium (Pl. XX, Fig. 3). Within the latter, sperms are formed which escape in the usual manner from a slightly exerted tube-like outgrowth immediately below the oogonial septa (Pl. XX, Fig. 4). In *M. macrandra* the first formed antheridia and oogonia are produced terminally on different hyphal branches (Pl. XX, Figs. 5, 6). As growth and reproduction proceed the diclinous habit is lost, and oogonia and antheridia occur in various positions. However, if both organs are formed on a single hyphal branch, alternating groups of one or the other type of structure are generally developed (Pl. XX, Fig. 25).

Occasionally one finds, particularly in *M. sphaerica*, smooth-walled, endogenous resting bodies (Pl. XX, Figs. 1, 29). Whether or not these are unfertilized oogonia which have developed parthenogenetically, as has been suggested, awaits further investigation. Lagerheim has described the formation of 'gemmae' in *M. polymorpha*. These consist of somewhat rounded chains of hyphal segments.

*Development of Gonapodya.*

No information concerning the early stages in the development of *Gonapodya* and its methods of attachment to the substratum are seemingly available. The writer has repeatedly endeavoured to determine the latter point, but without success. It can reasonably be supposed, however, that the development and anchorage are essentially similar to those of *Monoblepharis*. Work on these points, and especially the origin of the pseudo-septae, is greatly needed.

In its cytological aspects the sporangium of *G. prolifera* resembles that of *Monoblepharis*, according to Laibach. As has been previously pointed out in this paper, the type of zoospore and method of formation are also similar to *Monoblepharis*.

No confirmation of the sexual process described by Cornu (7), as occurring in *G. prolifera*, has as yet been published, and the nature of the sexual stage of the genus, if it exists, is still in doubt. Further details are included under the discussion of *G. prolifera*.

## TAXONOMIC TREATMENT.

Members of the two genera considered in this paper are found to be quite variable in nature, and it is frequently a difficult and puzzling matter to separate the species. For example, in even the most well-defined species of *Monoblepharis*, *M. sphaerica*, forms are occasionally found which nearly approximate those of *M. polymorpha* (Pl. XX, Fig. 28) and the closely related *M. macrandra*. Also, in the genus *Gonapodya*, variations and intergradations between the two species may often be so extensive as to make specific determination impossible.

Wide variations in size are also frequent, and often a single plant will bear organs approaching or equalling the recorded limits for the species. Variations in this respect are, therefore, not considered particularly significant.

*Monoblepharidales.*

Mycelium filamentous, the contents disposed in a reticulate or foamy manner; non-septate save when reproductive organs are formed, or possessing pseudo-septae. Non-sexual reproduction by means of posteriorly unciliate zoospores borne in sporangia; zoospores possessing an anterior group of refractive granules. Sexual reproduction, where known, by means of posteriorly unciliate antherozoids borne in antheridia, and non-ciliate oospheres borne in oogonia; the fertilized oosphere becoming a thick-walled oospore.

As originally conceived by Cornu, *Monoblepharis* (including *Gonapodya*), embraced saprolegniaceous organisms which possessed unciliate

zoospores. Indeed, with the advent of more accurate observations on the zoospores of various filamentous Phycomycetes, there can be little question that this idea was a correct one. The only other group of the aforementioned fungi possessing similar zoospores, the Blastocladales, seems closely related to the Monoblepharidales and, in the writer's opinion, might well be included in it.

*Monoblepharidaceae.*

Characters, those of the order.

Key to the Species of *Gonapodya* and *Monoblepharis*.<sup>1</sup>

Sexual reproduction unknown . . . . .	<i>Gonapodya</i> , <i>Monoblepharis</i>
Mycelium constricted by pseudo-septae . . . . .	<i>Gonapodya</i>
Sporangia long, tapering, siliquaeform . . . . .	<i>G. prolifera</i>
Sporangia more ovate in shape . . . . .	<i>G. polymorpha</i>
Mycelium without constrictions or pseudo-septae . . . . .	<i>Monoblepharis</i>
Sporangia narrowly cylindrical, always proliferating . . . . .	<i>M. regnensis</i>
Sporangia more ovate, rarely proliferating . . . . .	<i>M. ovigera</i>
Sexual reproduction known . . . . .	<i>Monoblepharis</i>
Oospores always endogenous (retained within oogonium), oogonia beaked, antheridia epigynous (borne on oogonium).	
Sex organs in fascicles . . . . .	<i>M. fasciculata</i>
Sex organs linearly arranged, basipetalous, plant very large	<i>M. insignis</i>
Oospores mostly exogenous (extruded from oogonium), rarely endogenous	
Antheridia usually hypogynous (beneath oogonium)	
Antheridia scarcely exerted, nearly always accom- panying oogonia . . . . .	<i>M. sphaerica</i>
Antheridia conspicuously exerted, in young plants occurring on separate branches from the oogonia, in older material in groups with the latter	
Oospores bullate . . . . .	<i>M. macrandra</i>
Oospores smooth . . . . .	<i>M. macrandra</i> var. <i>laevis</i>
Antheridia inserted on the oogonia (epigynous) . . . . .	<i>M. polymorpha</i>

*Monoblepharis* Cornu, Bull. Soc. Bot. France 18 : 59, 1871.

Mycelium non-septate, branched or unbranched, colourless, or with a slightly brownish tinge, attached by rhizoids to the substratum; content of hyphae disposed in a reticulate or foamy manner. Zoosporangia usually terminal, narrowly cylindrical or somewhat irregular in shape; cut off from the hyphae by cross walls; renewed by branching of the hyphae or by proliferation. Zoospores fully formed within the sporangium, escaping from the latter after the dissolution of its apex; posteriorly unciliate. Oogonia intercalary or terminal, usually narrowly pyriform to spherical, cut off from the attendant hyphae by cross walls; each exhibiting upon maturity a well-defined receptive papilla and a single egg. Antheridia variable in shape, usually somewhat cylindrical; variously placed; forming a small number of unciliate sperm entirely similar, save for their smaller

<sup>1</sup> This key presupposes that material has been subjected to conditions favourable for sexual and non-sexual reproduction.

size, to the zoospores. Oospheres after fertilization remaining in the oogonia or emerging, in either case developing into thick-walled oospores. Oospore upon germination producing a new thallus.

Syn. *Diblepharis* Lagerheim, loc. cit., p. 39.

*Monoblephariopsis* Laibach, loc. cit., p. 603.

The genus *Diblepharis* was established by Lagerheim to include *M. insignis* and *M. fasciculata* Thaxter, which were described as having biciliate zoospores. See, however, the remarks under the description of these species.

*Monoblephariopsis* was erected to include *M. regimens* Lag., a sporangial form differing from other species of *Monoblepharis* in having more slender hyphae, smaller sporangia which proliferated, and which lacked sex organs. As these differences seem only worthy of specific distinction from the sporangial stages of other species of the genus (which occasionally proliferate), and as no sexual stage has as yet been found with certainty, it has seemed better to replace this plant in *Monoblepharis* until further information on its life history is forthcoming.

*Monoblepharis sphaerica* Cornu, Bull. Soc. Bot. France 18:59, 1871 and Ann. Sci. Nat. V ser. Bot. 15:82, Pl. II, Figs. 1-6, 1872. Amend. Woronin, Mém. Acad. Imper. Sci. St. Petersb. 16:1-24, Pl. I, Figs. 1-16; Pl. II, Figs. 17-19, 21-7; Pl. III, Figs. 50-3, 1904.

(Pl. XX, Figs. 1-4, 29.)

Mycelium well developed, consisting of cylindrical, somewhat rigid, usually sparingly branched hyphae;  $7.5\ \mu$  in diameter in the stouter basal portions, tapering distally to  $2\ \mu$ . Sporangia narrowly cylindrical,  $72-104\ \mu$  in length by  $5.4-7.2\ \mu$  in diameter; borne singly in a terminal position or in groups. Zoospores  $5.4-9\ \mu$  in length by  $3.2-5.4\ \mu$  in diameter. Oogonia narrowly pyriform to sub-spherical, occurring singly, terminally, or in a linear series alternating with the antheridia;  $18-45\ \mu$  in length by  $8-19\ \mu$  tapering to  $5.4-16\ \mu$  in diameter. Antheridia narrowly cylindrical, hypogenous, opening by a slightly exserted tube formed just beneath the oogonial cross wall;  $9-18\ \mu$  in length by  $3.6-9\ \mu$  in diameter. Antherozoids 4-7 in number, about  $3.6\ \mu$  in length by  $1.5-2\ \mu$  in diameter. Oospores usually exogenous, thick-walled, brown,  $12.6-27\ \mu$  in diameter, covered by light yellow bullations  $1.5-2\ \mu$  in height. Germination of oospores not observed.

On submerged twigs of various types, leaves of conifers, animal material.

Occasionally, light brown, smooth-walled, endogenous resting bodies about  $28\ \mu$  long by  $15\ \mu$  in diameter are found (Pl. XX, Figs. 1, 29).

The oospores of Cornu's fungus, with one exception, were all endogenous. As was pointed out by Woronin, under favourable environmental conditions the oospores are exogenous. All gradations between endogenous and exogenous may be found in a single pustule of the fungus, and occasionally on a single plant.

*Geographic Distribution:* United States: New Hampshire; Hanover, *Sparrow* (S);<sup>1</sup> Mass: Waverley, Cambridge, *Sparrow* (S), Canton, *Linder* (F, S), Lexington, *Thaxter* (F); New York: Cold Spring Harbor (20), Ithaca, *Sparrow* (S); Penn.: Mt. Holly Springs, *Sparrow* (S). Europe: France, *Cornu*; Finland, *Woronin*; Germany, *Laibach*, Latvia, *Apinis* (1); England, *Barnes* and *Melville* (3), *Sparrow* (S.).

*M. polymorpha* Cornu, loc. cit., 1871, p. 59; loc. cit., 1872, p. 83, Pl. II, figs. 7-9; in Van Tieghem's 'Traité de Botanique' (1874 ed.), fig. 167 B, 4 (sporangia), and Fig. 167 C, 7, *l*, *m*, *n*, and 9.

Syn. *M. brachyandra* Lagerheim, Bihang Till K. Svensk. Vet.-Akad. Handlingar Afd. 3, 25:37, Pl. I, Figs. 1, 3, 5-10, 14-20, 35-45, 47, 52, 53, 55-62, 64-6; Pl. II, Figs. 6-10. 1900.

*M. brachyandra* var. *longicollis* Lagerh., loc. cit., p. 38, Pl. I, Fig. 53; Pl. II, Figs. 1-5.

(Pl. XX, Figs. 7-13, 19, 20, 28 (?), 36, 38, 39; Text-fig. 1, *a-m*, *r*; Text-fig. 2, *a-k*).

Mycelium filamentous, well-developed, consisting of somewhat rigid, cylindrical, rather frequently branched hyphae which, in their stouter, basal portions attain a diameter of 12-15  $\mu$ , tapering distally to 1.5-2  $\mu$ . Sporangia, narrowly cylindrical, rarely somewhat irregular, occurring singly, terminally, or occasionally in clusters sympodially arranged; 130-234  $\mu$  in length by 10.4-13  $\mu$  in diameter. Zoospores 10.4-13  $\mu$  in length by 7.8-10.4 in diameter. Oogonia in young material broadly to narrowly pyriform, in older plants becoming somewhat variable in shape, the even contour often being notched at the point of insertion of the antheridium; variable in size, usually 20-28  $\mu$  long, by 20-28  $\mu$  tapering proximally to 5-7  $\mu$  in diameter. Antheridia epigynous, often in a series of incompletely developed sex organs appearing hypogenous; when terminal, somewhat cylindrical; when intercalary, somewhat geniculate with a broadly conical apex; varying greatly in size, usually about 10-35  $\mu$  in length by 5-10  $\mu$  in diameter. Antherozoids 5-7 in number, each about 5.2  $\mu$  in length by 2.6  $\mu$  in diameter. Oospores spherical, nearly always exogenous; with a thick, brown wall beset with bullations 1.5-2  $\mu$  in height, or oftentimes with light coloured undulations; 12-25  $\mu$  in diameter. Oospore germinating by means of a hypha.

Saprophytic on submerged twigs of various types and animal remains.

<sup>1</sup> (S) = Writer's *Herbarium*. (F) = Farlow *Herbarium*.

After a study of many hundreds of specimens, the writer has not been able to separate clearly *M. brachyandra* from *M. polymorpha*. The former species is said to be distinct from *M. polymorpha*, chiefly in the uneven contour of the oogonium at the place of insertion of the shorter, stouter antheridium, the frequently intercalary position of the latter, or when epigynous, its tendency to be placed on the lower third of the oogonium. The oospores are said to differ in being of a smaller size, and in having flatter, broader bullations which may, in some cases be only slight undulations. Such differences are readily apparent when one has only a limited amount of material in early stages of development. However, if large amounts of the fungus are available, and the plants are observed over a long period of time, these differences become less distinct. One finds that, whereas in the first-formed sex organs the antheridia and oogonia are often disposed in a definite manner and possess definite, uniform shapes, in subsequent 'generations' of such organs formed on the same hypha, these characters vary considerably. Thus, one may find combinations of *polymorpha* and *brachyandra* characters on a single plant (Pl. XX, Figs. 13, 19, 20, 38). Such combinations are also evident in Lagerheim's figures of his species. The variety *longicollis*, based chiefly on slight differences in size, shape, and position of the sex organs and stronger bullations on the slightly smaller oospore is also subject to the afore-mentioned remarks. Variations are so marked in plants with a profuse development of sex organs that nearly every group of the latter might be considered a variety if based on such slight differences. Gemmae have been described by Lagerheim as occurring in this species.

As originally described by Cornu, *M. polymorpha* was sufficiently inclusive to embrace *M. macrandra*.

*Geographic Distribution*: U.S.: New Hampshire, Hanover, *Sparrow* (S); Mass. Cambridge, Lexington, *Thaxter* (F); New York, Ithaca, *Sparrow* (S). Europe. France, *Cornu*; Germany, *Claussen* (4), *Minden* (15), *Laibach*; Switzerland, *Tiesenhausen* (23); Sweden, *Lagerheim*; Denmark, *Petersen* (18); Finland, *Woronin*; Latvia, *Apinis*; Austria, *Wettstein* (25).

*M. macrandra* (Lagerheim) *Woronin*, loc. cit., p. 13, Pl. II, Figs. 32-46; Pl. III. Figs. 47-9, 54-70.

Syn. *M. polymorpha* var. *macrandra* Lagerh., loc. cit., p. 35, Pl. I, Figs. 2, 4, 21-4, 36-46, 48-51, 54, 63, 67, 68; Pl. II, Figs. 11-26.

*M. polymorpha* *Cornu* pro parte, loc. cit., 1872, p. 84, Pl. II, Figs. 10-32, and Van Tieghem's 'Traité de Bot.' (1874 ed.), Figs. 167, C, 7, *p*, *q*.

(Pl. XX, Figs. 5-6, 25, 31; Text-fig. 1, *s*, *t*, *u*.)

Mycelium filamentous, exceedingly well-developed, consisting of rather flexuous, nearly isodiametric, profusely branched hyphae which under



excellent conditions for growth may interlock and completely envelop the substratum; hyphae  $5\mu$ , tapering distally to  $1.5\text{--}2\mu$  in diameter; bearing occasional irregular swellings. Sporangia narrowly cylindrical,  $45\text{--}130\mu$  in length by  $4.5\text{--}6\mu$  in diameter; occurring singly at the tips of the hyphae or grouped in sympodial or fasciculate fashion; occasionally proliferating. Zoospores  $7.8\mu$  in diameter by  $9\text{--}12\mu$  in length. Oogonia broadly cylindrical to narrowly pyriform, at first formed singly in a terminal or intercalary position, later occurring sympodially or more commonly in fascicles associated with antheridia. The latter cylindrical, at first formed at the tips of hyphal branches other than those bearing oogonia, later occurring with them; always strongly exserted; variable in size, usually about  $25\text{--}35\mu$  long by  $5\text{--}7\mu$  in diameter. Antherozoids  $5\text{--}14$  in an antheridium; about  $6\mu$  in length by  $4\mu$  in diameter. Oospores normally exogenous, having a tendency to fall away from the oogonium;  $13\text{--}25\mu$  in diameter, the brown wall covered by lighter coloured bullations  $1.5\text{--}2\mu$  in height. Germination of oospores not observed.

Saprophytic on submerged twigs of various types.

There seems little question that Cornu observed this species if one combines the description of variations found in *M. polymorpha* given at the top of page 84 of his monograph with the figures shown by him in this paper and in Van Tieghem's 'Traité de Botanique'. Lagerheim considered this species to be a variety of *M. polymorpha*, but from the position of the antheridium, its strong exsertion, the tendency of one or other of the sex organs to be formed in groups, there is little chance of confusing this species with others of the genus. However, forms are occasionally found which produce antheridia approximating those of the closely related *M. sphaerica* (Pl. XX, Fig. 5), and rarely epigynous ones are observed. Whether or not these variations are due to hybridism, as has been suggested, awaits further study.

A form resembling *M. macrandra* in the disposition of the sex organs, but differing from it in the possession of dark brown, smooth-walled oospores,  $25\mu$  in diameter, was found at Ithaca, N.Y. (Pl. XX, Figs. 14–16). Isolated instances of smooth-walled oospores may be occasionally observed in species normally possessing bullate ones, but in the present material the former type was exclusively formed. A further characteristic of these oospores was the presence, seemingly in the content, of regularly placed, minute oil globules. While this fungus may be found in the future to be worthy of specific rank, significant phases in its life-history remain yet to be observed, and it is therefore termed *M. macrandra* var. *laevis* n.var. for the present. It was found saprophytic on submerged rose fruits.

*Geographic Distribution*: U.S.: New Hampshire, Hanover, Sparrow (S); Maine, Kittery Pt., Thaxter (F. as *M. polymorpha*); New York, Ithaca, Sparrow (S). Europe: France, Cornu (as *M. polymorpha*); Sweden, Lagerheim; Finland, Woronin;

Switzerland, *Tiesenhausen*; Denmark, *Petersen*; Germany, *Laibach*, *Minden* (16); Hungary, *Scherffel* (19); England, *Sparrow* (S), *Barnes and Melville* (loc. cit.), as *M. polymorpha*; Austria, *Wettstein* (loc. cit.).

*M. insignis* Thaxter, Bot. Gaz. 20:438, Pl. XXIX, Figs. 1-7, 1895.

Syn. *Diblepharis insignis* (Thaxter) Lagerheim, loc. cit., p. 40.

(Pl. XX, Fig. 17).

Hyphae straight, rigid, hyaline, or very pale reddish brown, nearly cylindrical, rarely branched, 1.5-2.5 mm. in length by 8-15  $\mu$  in diameter. Antheridia broad, subconical to subcylindrical, straight or slightly divergent, the rounded tip often bent slightly inwards, nearly symmetrical or often with base irregularly protruded on its inner side. Antherozoids numerous (about 24-32) 1-ciliate. Oospores maturing within the oogonium, smooth, pale amber-brown, spherical to long oblong or irregular in outline, 30-45  $\times$  22-33  $\mu$ . Oogonia single or several superimposed at the tips of the hyphae, irregular in form. Zoosporangia rare, similar to the oogonia; zoospores 2-ciliate (?), about 10-12  $\mu$  in diameter.

On submerged sticks in pools and ditches.

(Adapted from Thaxter.)

*Geographic Distribution*: U.S.: Mass., Weston, Medford, *Thaxter* (F); Maine, Kittery Pt., *Thaxter* (F).

The type material of this and the following species (*M. fasciculata*), excellently preserved in glycerine mounting fluid, were examined at the Farlow Herbarium. No further points of morphological interest not covered by Dr. Thaxter's paper and figures were observed.

Presumably, the zoospores described and figured for this species were not seen to emerge, hence the ciliation is doubtful. A further discussion of the zoospores is given under the following species.

This and *M. fasciculata* are indeed remarkable members of the genus. In their relatively enormous size, slight brown coloration of the protoplasm, process of fertilization involving a discharge of a substance from the oogonium apparently attractive to the sperm, and smooth, endogenous oospores, they are strikingly different from other species of the genus. In connexion with Laibach's statement, that he regarded the endogenous oospores of these two species as due to poor environmental conditions, the following facts might be pointed out. It is true in other, exogenous, species of *Monoblepharis*, that sexual reproduction occurring under unfavourable environmental conditions does result in the formation of many endogenous oospores. However, in such cases there are always some exogenous examples under even the poorest of conditions. In an examination of Professor Thaxter's slides of his species, containing many plants from three localities, over 80 oospores were observed, none of which was exogenous.

Hence, until further collections of material indicate otherwise, endogenous oospores must be regarded as typical for these two species.

*M. fasciculata* Thaxter, loc. cit., p. 439, Pl. XXIX, Figs. 8-12.

Syn. *Diblepharis fasciculata* (Thaxter) Lagerheim, loc. cit., p. 40.

(Pl. XX, Fig. 18).

Hyphae straight, rigid, cylindrical, simple, or rarely branched, except at the tips, 1-2 mm. long by  $6\ \mu$  in diameter. Antheridia narrow, tapering slightly, straight, not divergent. Antherozoids about 16 in an antheridium,  $3\ \mu$  in diameter. Oogonia evenly oval oblong or elliptical, the neck small and prominent, usually shorter than the antheridium which is always present, single and terminal or borne superimposed on short crowded branches from the tips of the fertile hyphae. Oospores more or less regularly oval oblong or elliptical, smooth, pale amber-brown, maturing within the oogonium,  $22 \times 18\ \mu$ . Zoosporangia like the oogonia, bearing antheridia; the zoospores 2-ciliate, about  $5-6\ \mu$  in diameter.

On submerged sticks with the last (*M. insignis*).

(Adapted from Thaxter.)

*Geographic Distribution*: U.S.: Mass., Weston, Medford, Thaxter (F).

This species is 'distinguished from the last (*M. insignis*) by its constantly smaller size and the greater regularity and different form of its sexual organs, as well as by its fasciculate habit'.

The question of the ciliation of the zoospores in this species as well as in *M. insignis* is in need of further investigation. The biciliate bodies, borne in organs similar to oogonia, are very different in shape and much too small in comparison with the sperm to be consistent with what is found in other species of the genus. While it must be admitted that Thaxter's two species are anomalous in other respects as well, and that these 'anomalies' (and even the biciliate spores) may be seen in the actual specimens at the Farlow Herbarium, the fact that similar biciliate bodies were also found by him (22) in species known, not only from the writer's observations, but from those of Cornu, Lagerheim, Woronin, and Laibach as well, to possess more cylindrical, unciliate spores, would seem to indicate that the biciliate bodies were, as has been suggested, extraneous, parasitic organisms. This interpretation seems further borne out by the constant presence in such oogonia of residual oil globules. The latter might well be explained as ooplasmic material unavailable for the parasite. From personal conversation with Professor Thaxter concerning the question of zoospores in this genus, it was obvious that he regarded as antheridia the cylindrical structures considered by other investigators to be sporangia. However, if the latter are antheridia and they can be produced and

maintained at will, to the exclusion of oogonia—as has been shown to be the case by both Laibach and the writer—it is difficult to understand what organs the thousands of free-swimming bodies produced by this method could fertilize. A final disposition of the matter in Thaxter's two species cannot be made until more material is collected and examined with this point in mind.

#### SPECIES IMPERFECTLY KNOWN.

*Monoblepharis regignens* Lagerheim, loc. cit., p. 39, Pl. I, Figs. 11–13.

Syn. *Monoblephariopsis regignens* (Lagerh.) Laibach, Jahrb. wiss. Bot. 66: 603, Text-fig. 4, Pl. XII, Figs. 20–27, 1927.

(Pl. XX, Fig. 26.)

Mycelium exceedingly tenuous, sparingly branched; hyphae 1.8–2  $\mu$  in diameter, about 5  $\mu$  at the base. Sporangia narrowly cylindrical, but distinctly broader than the hyphae on which they are usually terminally placed; 18–36  $\mu$  in length by 5.4–7.2  $\mu$  in diameter; new sporangia formed by proliferation partially or wholly through the apex of the old one, or occasionally by cymose branching. Zoospores 8  $\mu$  long by 5  $\mu$  in diameter. Sexual reproduction not observed.

Saprophytic on submerged twigs of various types.

See remarks under the generic description of *Monoblepharis*.

*Geographic Distribution*: U.S.: New Hampshire, Hanover, *Sparrow* (S); Mass., Arlington, *Sparrow* (S); Maine, Kittery Pt., *Thaxter* (F); New York, Ithaca, *Sparrow* (S). Europe: Sweden, *Lagerheim*; Germany, *Laibach*; England, *Sparrow* (S).

*M. ovigera* Lagerheim, loc. cit., p. 39, Figs. 69–70.

(Pl. XX, Figs. 23, 33, 35, 37.)

Mycelium often profuse, composed of very delicate, occasionally branched hyphae 3–4  $\mu$  in diameter. Zoosporangia terminal or intercalary, ovoid; 10–13  $\mu$  in diameter by 23–33  $\mu$  in length; rarely proliferating. Zoospores often formed in two rows; 8  $\mu$  long by 6  $\mu$  in diameter. Sexual reproduction not observed.

Saprophytic on submerged twigs of various types.

In connexion with the lack of a sexual stage, the writer wishes to call attention to certain structures found by him among material of this species collected at Ithaca, N.Y. These bodies, figured in Pl. XX, Figs. 21, 22, 24, 27, were found among filaments and sporangia of *M. ovigera* (Pl. XX, Figs. 33, 35, 37). In their shape and arrangement they strongly resemble the sexual apparatus of *M. polymorpha*, although considerably smaller in size. Near these, colourless as well as brown, smooth-walled resting bodies were observed (Pl. XX, Fig. 22). As the amount of material was exceedingly scanty, and as cysts of various unicellular organisms are frequently present

in such cultures, the writer hesitates to regard the two as integral parts of the same organism. Further, he has not found with certainty any organic connexion between the sporangial stage of *M. ovigera* and this supposed sexual stage. These observations are presented here with the hope that they will be of interest to other investigators of the group, and that their significance and relationships will become clearer with subsequent collections of the species.

*Geographic Distribution:* U.S.: New Hampshire, Hanover, *Sparrow* (S); New York, Ithaca, *Sparrow* (S). Europe: Sweden, *Lagerheim*; England, *Sparrow* (S).

*Gonapodya*, Fischer, Rabenhorst, Kryptogamenfl. 1:4:382, 1892.

Mycelium irregularly or dichotomously branched, varying in extent of development, often profuse; method of attachment to substratum not observed; composed of cylindrical, often moniliform hyphal segments delimited by hyaline pseudo-septae, which are often accompanied by constrictions; hyphae with a reticulate or foamy, or sometimes homogeneous, disposition of the protoplasm. Zoosporangia terminal, occurring singly or in fascicles; proliferous; varying in shape. Zoospores completely formed within the sporangium, escaping upon the deliquescence of the apex of the latter; ovoid to cylindrical; posteriorly uniloculate, possessing an internal organization similar to that of *Monoblepharis*. Sexual reproduction, according to Cornu, by means of oogonia and antherozoids.

When more is known about the sexual stage of this genus it may be more properly placed in another order, perhaps the *Blastocladales*. However, in view of the vacuolization of the protoplasm, the formation, discharge and structure of the zoospores, and the account of that excellent observer Cornu, of a sexual stage, the writer is inclined in this matter to follow Fischer, Schröter, and Laibach, at least for the present.

*Gonapodya prolifera* (Cornu) Fischer, loc. cit., p. 382.

Syn. *Monoblepharis prolifera* Cornu, loc. cit., 1871, p. 59; 1872, p. 16, and Van Tieghem, 'Traité de Bot.' (1874 ed.), Fig. 167, B, 5.

*Saprolegnia siliquaeformis* Reinsch, Jahrb. wiss. Bot. 11:293, Pl. XV, Figs. 12-13, 1876.

*Gonapodya siliquaeformis* (Reinsch) Thaxter, Bot. Gaz. 20:480, Pl. XXXI, Figs. 6-10, 1895.

(Pl. XX, Figs. 30, 32.)

Mycelium composed of hyphae more or less regularly divided by pseudo-septae into short, elliptical to long clavate segments; copiously and successively sub-umbellately branched, the branches diverging in a dense tuft from a common base. Sporangia once to many times proliferous, the secondary sporangia only slightly exserted; long pod shaped,

inflated below, the sometimes very elongate distal portion tapering gradually to a blunt apex; borne sessile on the terminal cell of a branch or separated from it by a clearly defined constriction. Zoospores variable in number, up to fifty or more in a sporangium; posteriorly uniciliate, elliptical, or somewhat cylindrical. Sexual reproduction by means of oogonia and antherozoids; oospores oval in terminally perforate oogonia like the sporangia (Cornu).

(Modified from Thaxter.)

On decaying fruits and twigs of various types in water.

This is a rather common and variable species, generally found in small white pustules on rosaceous fruits under very foul environmental conditions. Variations in the shape of the sporangium are extensive and may approach those typically found in the following species (*G. polymorpha*).

Cornu reported (7) that in his fungus he found oval, colourless oospores borne in oogonia similar in shape to zoosporangia and which were produced from an oosphere fertilized by a sperm. No further information or figures were given. Thaxter has suggested that Cornu mistook unusually small zoospores for antherozoids, and that his oospores were merely encysted, secondary sporangia. In this connexion the writer would like to point out certain thick-walled, encysted structures recently found by him in very old cultures of the fungus on apple (Pl. XX, Fig. 30). Each seems to be simply a single, secondary sporangium which, due to the action of unfavourable environmental conditions, has encysted. It is entirely possible that the two species of *Gonapodya* lack a sexual stage, and that Cornu's 'oospore' was some sort of encysted sporangium similar to the aforementioned one. However, considering the small amount of investigation that this group has thus far been accorded, it seems quite useless to venture any opinion now concerning the matter.

*Geographic Distribution:* U.S.: Mass., Cambridge, *Sparrow* (S), *Thaxter* (F); New Hampshire, Hanover, *Sparrow* (S); Maine, Kittery Pt., *Thaxter* (F); New York, Cold Spring Harbor, *Sparrow* (S) (loc. cit.); Ithaca, *Sparrow* (S); Michigan, Ann Arbor, *Kanouse* (10). Europe: France, *Cornu*; Germany, *Reinsch*, Minden (17), *Laibach*; Denmark, *Petersen*; Bulgaria, *Valkanov* (24); Latvia, *Apinis*; England, *Sparrow* (S), *Barnes* and *Melville*.

In calling this plant *G. prolifera*, rather than the more accepted binomial *G. siliquaeformis* (Reinsch) Thaxter, the writer is guided by the following facts.

In 1871 Cornu briefly but clearly characterized the genus *Monoblepharis*. Included in this description was a rather detailed account of the non-sexual stage and zoospore as well as the sexual process. At that time three species were defined in the following order: *M. prolifera*, *M. sphaerica*, and *M. polymorpha*. The first of these was defined as having proliferous sporangia and no sex organs, while the others were differentiated

on the relative positions of the antheridia and oogonia. Essentially the same account of *M. prolifera* was given in Cornu's 1872 paper (p. 16), but the form was not illustrated. In the 1874 edition of Van Tieghem's translation of Sach's 'Lehrbuch der Botanik', considerable original material was incorporated by various authors. Of these, Cornu contributed sixteen figures of various aquatic Phycomycetes, none of which, with the exception of Figs. 167 A, 1 and 167 C, 6, was included in his monograph. In Fig. 167 B, 5, there appears a recognizable and convincing figure of his *M. prolifera* which leaves no doubt, at least in the writer's mind, that this fungus was entirely similar to Reinsch's *Saprolegnia siliquaeformis* described two years later. The writer cannot, therefore, concur with Thaxter in concluding that no recognizable figure or description had been associated with the name *M. prolifera* and hence, that Reinsch's specific designation should be accepted.

*G. polymorpha* Thaxter, Bot. Gaz. 20:481, Pl. XXXI, Figs. 11-16, 1895.

(Pl. XX, Fig. 34.)

Hyphae irregularly or more frequently dichotomously branched, more or less regularly divided into short oval, or irregular segments, the segmented portion arising directly from the substratum or more often confined to tufts of branchlets borne sub-umbellately on the ends of slender, elongate hyphae in which the segmentation is indistinct or obsolete; the segmentation frequently ill-defined or obsolete throughout the whole vegetative body. Sporangia variable in size and form, long oval, tapering rather abruptly to the blunt tip; terminal and solitary or sometimes several arising from a single segment; once to many times proliferous, the hyphae sometimes traversing and growing beyond the empty sporangium. Zoospores somewhat variable in size and number, usually about  $13\mu$  long by  $7\mu$  in diameter. Sexual stage unknown.

(Modified from Thaxter.)

On submerged fruits of various types, especially those of the Rosaceae; submerged twigs of fir, spruce mucilage, and twigs of deciduous trees.

The general habit of this species is more open and ramose than that of *G. prolifera*. It is usually found under less foul environmental conditions than the last-named species. Whether or not the difference in environment is responsible for the differences in the two species awaits further investigation.

*Geographic Distribution*: U.S.: New Hampshire, Hanover, *Sparrow* (S.); New York, Cold Spring Harbor, *Sparrow* (S.), Ithaca, *Sparrow* (S.); Mass., Maine, Kittery Pt., *Thaxter* (F.). Europe: Denmark, *Petersen*; Latvia, *Apinis*; Germany, *Minden*; England, *Barnes* and *Melville*, *Sparrow* (S.)

Thaxter has described oospores about  $54\mu$  in diameter with laminated,

refractive walls about  $18\mu$  thick, as often occurring with *G. polymorpha*, although he was not successful in demonstrating any definite connexion between the two. These bodies have been considered by Minden (17) to be the oospores of his *Pythiogeton utriforme*. Petersen (18) has reported encysted zoospores, termed 'resting spores', in the sporangia of this species. As in the preceding species, it is probable that the sexual stage of this fungus has not yet been observed.

#### DISCUSSION.

The members of the Monoblepharidales present a number of points of general mycological interest.

Since the discovery of *Monoblepharis*, its origin and relationships have intrigued the minds of speculative mycologists perhaps more than any other fungus. The obvious resemblance of its sexual reproduction to that of certain algae has been emphasized by those who consider the fungi, and particularly the Phycomycetes, as degenerate algae. Of the algae, *Monoblepharis* is said to have the greatest affinities with *Oedogonium* or *Vaucheria*. Atkinson (2) has reviewed the more significant papers dealing with this point and there is no need to include them here. Cornu himself, while suggesting affinities with 'les Coléochétées' and *Oedogonium*, pointed out that the differences were very great and that this analogy was of little weight. Later observers of the fungus have also been impressed with its similarity either to *Oedogonium* (Lagerheim) or to *Vaucheria* (Thaxter, Laibach). Cytologically, the sexual process of the fungus is seemingly more like that of *Oedogonium* than of *Vaucheria*. Morphologically, however, it exhibits such marked differences from these algae, especially with respect to the shape and ciliation of the zoospore, that any theory of its derivation from these forms is beset with difficulties. Other phylogenists have considered *Monoblepharis* to be derived from some lower group of fungi possessing isogamous planogametes. This view has certain points in its favour. The motility of all three types of uninucleate reproductive structures in most species of *Monoblepharis*, namely, the zoospore, antherozoid, and oosphere, is certainly of some significance. Further, the fact that one of these gametes, the egg, is appreciably modified would seem to argue that this fungus is not the starting-point of a developmental series, but rather an intermediate one. This is further borne out by the recent discovery by Kniep (11) of the anisogamous planogametic reproduction of *Allomyces*. Further investigation will no doubt reveal other filamentous fungi which will possess isogamous planogametic reproduction and which may also indicate what line of development the thallus has undergone. Perhaps these forms will justify the theory of the algal relationship of *Monoblepharis*; perhaps they will indicate a connexion with some chytridiaceous ancestor having isogamous reproduction, but, until we



find out what forms actually occur in nature, the futility of any opinion on the matter is obvious.

Another point of interest is the supposed rarity of these forms. This idea is no doubt due to the lack of general knowledge concerning the methods of collection, and particularly the treatment of material after collection. These phases have been discussed in a previous part of the paper. It is interesting to note that all the members of the order collected by the writer, with the exception of *M. macrandra* var. *laevis*, were obtained in a single small spring at Hanover, New Hampshire, on birch twigs. The frequency of occurrence of these fungi is undoubtedly greater than has been supposed in the past, and it is hoped that the present paper will assist somewhat in stimulating an intensive investigation of the points of more fundamental interest so admirably displayed by these organisms.

#### SUMMARY.

The first part of this paper includes an historical account of a small order of aquatic Phycomycetes, the Monoblepharidales, the methods of collecting them, and a description of their development, structure, and life-history. The thallus of *Monoblepharis*, produced from the germinating zoospore, is filamentous and is attached to the substratum by rhizoids. The plant bears, usually at its tip, the reproductive organs. These are of three types: zoosporangia, bearing posteriorly uniciliate zoospores; antheridia, containing antherozoids which are entirely similar to the zoospores but smaller; oogonia, each containing a single, uninucleate egg. After fertilization by a single antherozoid, the egg, in most species, emerges from the oogonium and develops into a thick-walled oospore. Karyogamy occurs during the formation of the oospore wall (Lagerheim, Laibach). The oospore germinates after a period of rest by means of a germ tube. Meiosis occurs at some time during this process, the first division of the resting nucleus probably being heterotypic (Laibach). Early stages in the development of *Gonapodya* are not known. The mature plant is similar to that of *Monoblepharis*, but, in contrast to the latter, the mycelium possesses pseudo-septae. Sporangia, bearing zoospores similar to those of *Monoblepharis*, are the only reproductive structures known with certainty in *Gonapodya*. Encysted sporangia are occasionally found.

In the second part of the paper a taxonomic account of the species based, save in two instances, on living material collected in various parts of the eastern United States and at Cambridge, England, is given. One new variety, *Monoblepharis macrandra* var. *laevis*, is described. A brief account of certain structures resembling sex organs, found among material of *M. ovigera*, is given, and the paper concludes with a brief discussion of the phylogeny and distribution of the order.

In conclusion, the writer wishes to thank the Board of Trustees of Dartmouth College, Hanover, N.H., for their kindness in extending his leave of absence from teaching duties so as to facilitate the completion of this and other papers. He is also greatly indebted to the late Professor Roland Thaxter for information concerning the habits and methods of collection of these fungi. It is also a pleasure to acknowledge his indebtedness to Professor W. H. Weston, Jr., of Harvard University, and Mr. F. T. Brooks, of Cambridge University, for extending to him the facilities of their laboratories and for their advice and helpful criticism.

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## EXPLANATION OF PLATE XX.

Illustrating Dr. F. K. Sparrow's paper on 'The *Monoblepharidales*'.

All figures were drawn with the aid of the camera-lucida. The approximate magnifications are given in each instance.

Figs. 1-19, all  $\times 375$ .

Fig. 1. Tip of filament of *Monoblepharis sphaerica*, showing fascicle of reproductive organs which includes two rather large, cylindrical sporangia, a smooth-walled 'chlamydospore', exogenous and endogenous oospores, and empty, hypogenous antheridia.

Figs. 2-4. Stages in the development of the sex organs of *M. sphaerica*. The antheridium of this species is hypogenous.

Fig. 5. Habit of a fruiting tip of *M. macrandra*. In younger stages of development, the antheridia and oogonia are usually borne on separate branches of the mycelium; in this case, two antheridia are so borne, but a third is hypogenously placed; however, it is, in contrast to *M. sphaerica*, strongly exerted.

Fig. 6. Series of oogonia of *M. macrandra* being formed in basi-petalous succession. Very typical of this species.

Fig. 7. Complete plant of *M. polymorpha*, killed and fixed and stained with cotton blue. The rhizoidal system, vegetative nuclei in axis of plant, larger egg nucleus and mature oospores are shown. The antheridia in this species are typically epigynous, i.e. inserted on oogonium.

Figs. 8-12. Stages in the development of the sex organs of *M. polymorpha*. The antheridium, in this species, is first formed. The antheridium of Fig. 12, second oogonium, is not visible in this view.

Fig. 13. A plant of *M. polymorpha*, showing combination of *polymorpha*, *brachyandra* and even *sphaerica*, antheridial characters.

Figs. 14-16. *M. macrandra* var. *laevis* n.var. Plants showing disposition of sex organs and smooth-walled exogenous oospores.

Fig. 17. Fruiting tip of *M. insignis*, showing arrangement of sex organs, and endogenous, smooth-walled oospores. Note the large size of this species as compared with the others drawn to the same scale. From a glycerine mount of type material.

Fig. 18. *M. fasciculata*. Fruiting tip showing arrangement of sex organs. The fasciculate habit is not so pronounced in this specimen as in others found on the same slide and illustrated by Dr. Thaxter. From glycerine mount of type material.

Fig. 19. A fruiting tip of *M. polymorpha* showing a short, blunt, antheridium inserted in an oogonal notch on the upper third of the oogonium, and an oospore possessing undulate walls; a combination of *polymorpha* and *brachyandra* characters.

The absolute scales of Figs. 24 ( $\times 600$ ) and 32 ( $\times 285$ ) are drawn along side of them; the scale for all other figures is drawn near Fig. 27 ( $\times 375$ ).

Fig. 20. Habit of fruiting tip of *M. polymorpha*, showing arrangement of sex organs and variations in the bullation of the oospores.

Fig. 21. Possible sex organs of a *Monoblepharis*; associated with sporangia of *M. ovigera*.

Fig. 22. One of many similar free-floating resting bodies found near the structures shown in Fig. 21.

Fig. 23. Proliferated sporangia of *M. ovigera*.

Fig. 24. Another type of structure of possible sexual significance found among filaments of *M. ovigera*. Note scale of figure.

Fig. 25. Habit of well developed fruiting tip of *M. macrandra*, showing groups of rather large sex organs.

Fig. 26. Proliferated sporangia of *M. regimens*, the terminal of one which shows the zoospores being discharged.

Fig. 27. A third type of fruiting structure found in the same pustule as that of *M. ovigera*.

Fig. 28. A fruiting tip which combines the antheridial characters of *M. polymorpha* and *M. sphaerica*.

Fig. 29. A typical fruiting tip of *M. sphaerica* showing a somewhat different type of 'chlamydo-spore' from that shown in Fig. 1, as well as an elongate oogonium, and oospores.

Fig. 30. *Gonapodya prolifera*. A type of irregular, encysted sporangium, sometimes found in old cultures.

Fig. 31. *M. macrandra*. Habit of fruiting tip.

Fig. 32. *G. prolifera*. Portion of a plant, showing hyphal links and proliferated sporangia.

Fig. 33. *M. ovigera*. Immature sporangium.

Fig. 34. *G. polymorpha*. Portion of a plant showing a discharging sporangium, and proliferated ones.

Fig. 35. *M. ovigera*. Mature sporangium with zoospores ready for discharge.

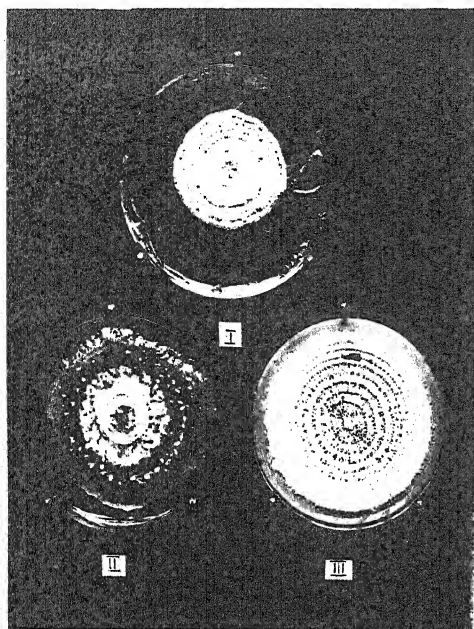
Fig. 36. *M. polymorpha*. Plant bearing short, stout antheridia.

Fig. 37. *M. ovigera*. Immature slightly tilted sporangium. This tilting is very generally found in this species.

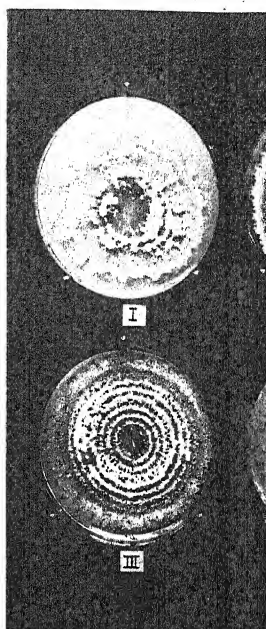
Fig. 38. Fruiting tip of what is possibly *M. polymorpha*, possessing broad conical, apical antheridia, smooth-walled, endogenous oospores as well as an exogenous one with an undulating wall.

Fig. 39. Germinating oospore of *M. polymorpha*.

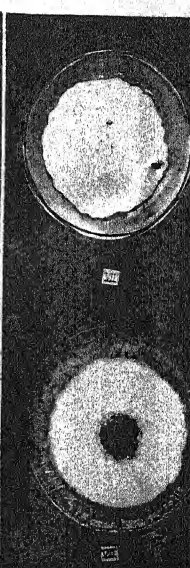
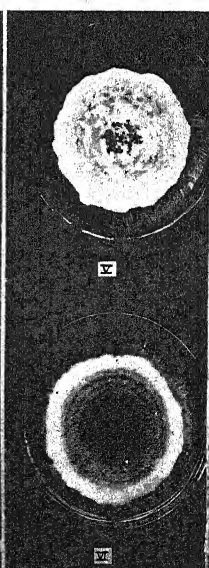
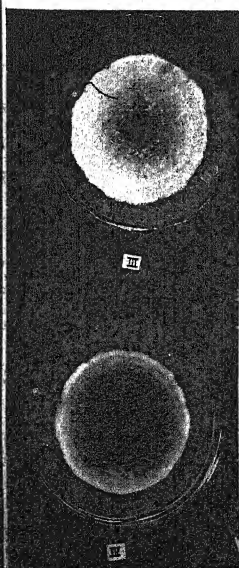
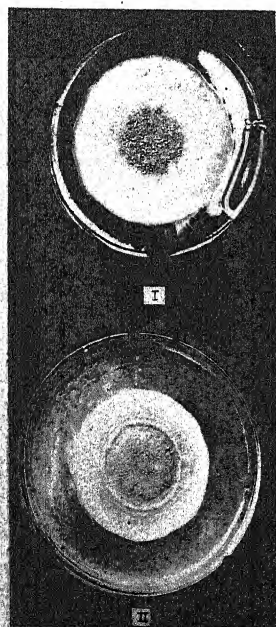




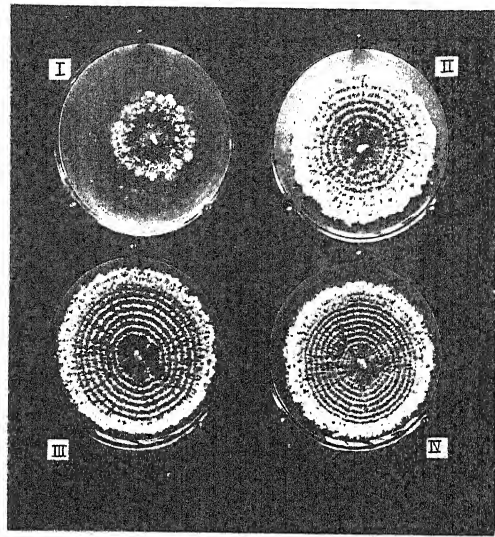
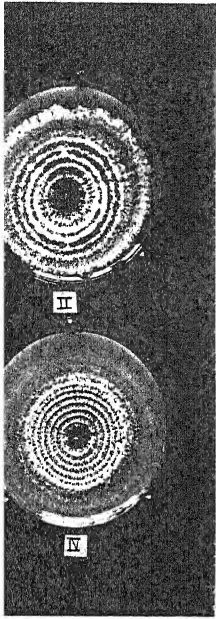
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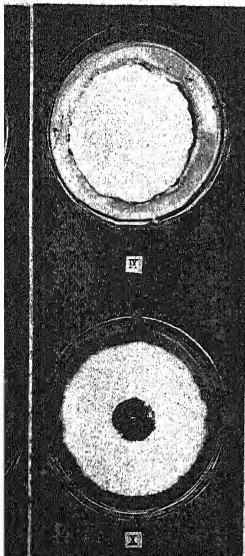
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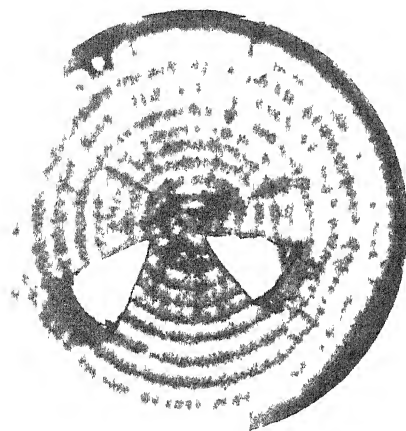


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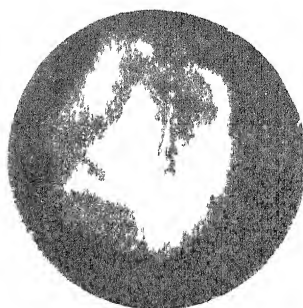


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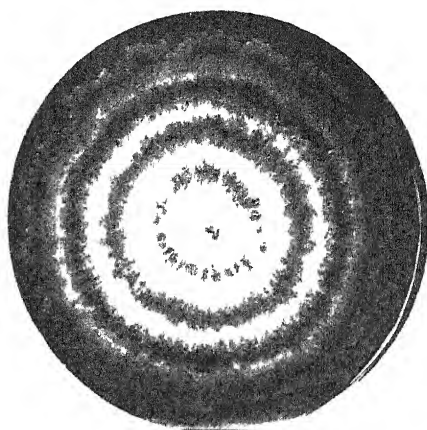




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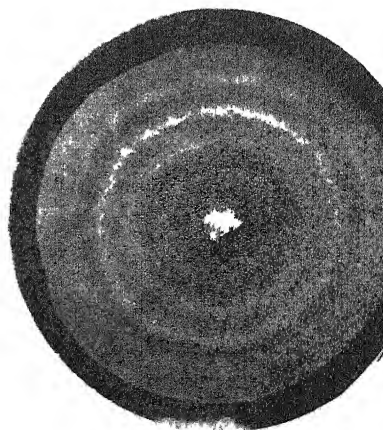
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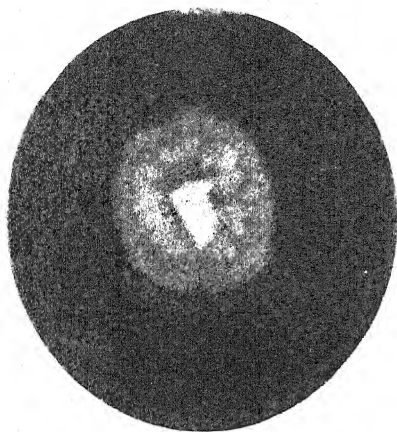
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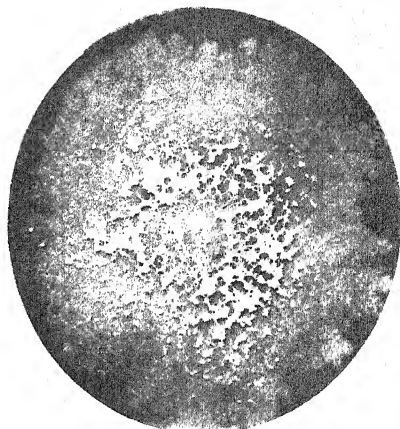
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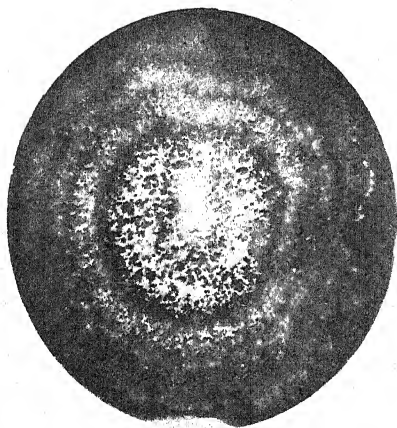




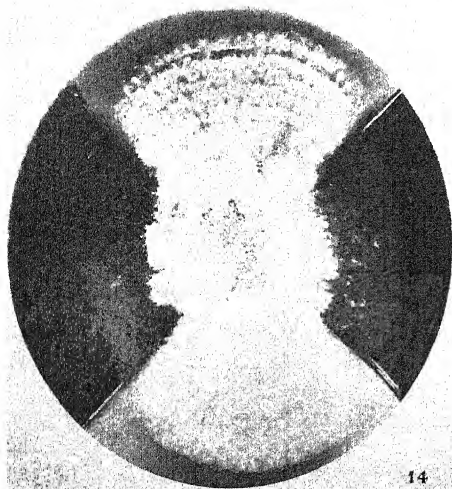
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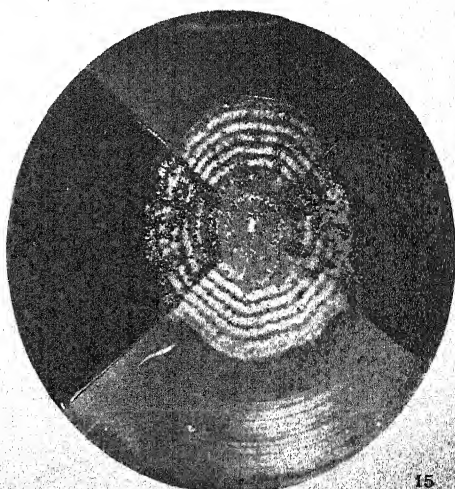
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# On the Preservation of Fungi.

BY

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With six Figures in the Text.

FUNGI are one of the most difficult groups of plants to preserve as satisfactory specimens either for museums, for class work, or for herbaria. They can, of course, be preserved in glass jars in spirit or formalin, but the specimens are bulky, take up much shelf-space, and in large quantity are costly. In addition, they are unsuitable for handling or close examination unless the risk is taken of destroying the specimen.

For some time attempts have been in progress to find a satisfactory way of preserving fungi that would leave their shape unaltered, would be cheap, and would give specimens that could be handled and stored in a small space. As some degree of success has been obtained, it may be of interest to detail the various methods employed and the advantages and disadvantages of each.

*Fossilizing specimens.* Various attempts were made to mineralize fungi and thus obtain fixed specimens. Dropping a solution of calcium bicarbonate on fungi sometimes gave a chalky cast of the specimen, but the deposit was usually mainly superficial and the softer fungi decayed long before any fossilization had taken place. Better results were obtained by using strong solutions of calcium chloride and of a mixture of sodium carbonate and sodium phosphate. The fresh specimens were placed first in the calcium chloride for two days and then for two days in the carbonate-phosphate mixture, after first washing the calcium chloride from the surface. Frequent transference from one solution to the other was required before the specimens were calcified right through. Any deposit on the surface can be removed by dipping in hydrochloric acid and washing before drying. A photograph of calcified *Coprinus* sporophores prepared in this way is shown in Fig. 1. The method is, however, very tedious.

*Cellulose acetate.* A further attempt in the preservation of specimens of delicate fungi was made by using cellulose acetate as an impregnating material. The fungi were dehydrated in spirit followed by absolute alcohol,

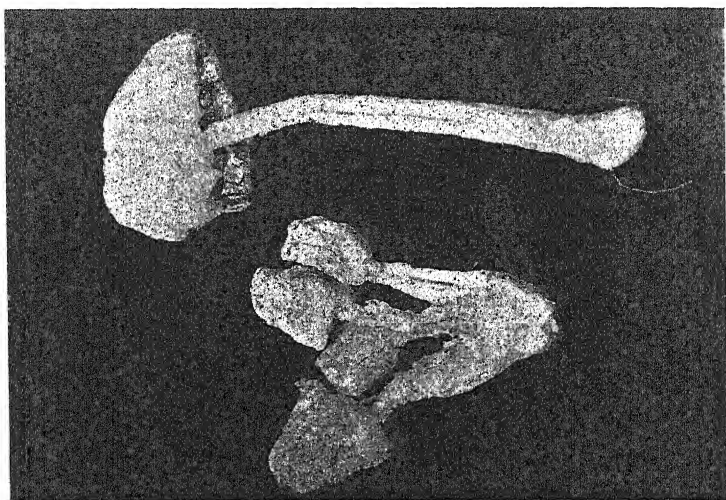


FIG. 1. Artificially calcified *Coprinus* sporophores.

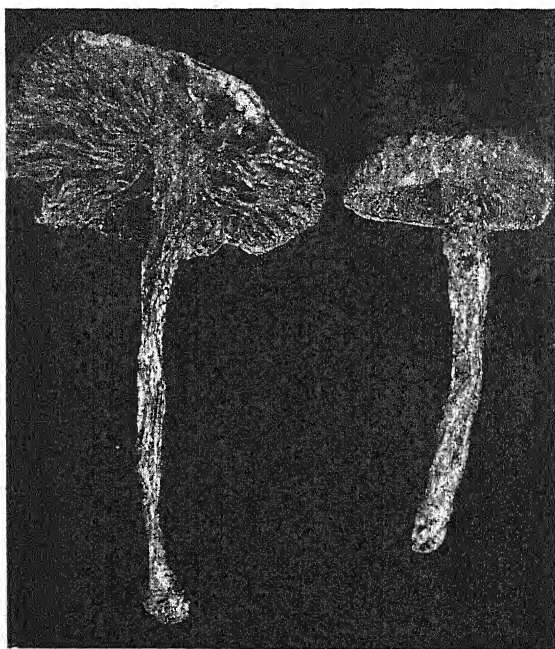


FIG. 2. *Collybia* sporophores impregnated with cellulose acetate.

and then passed through acetone until the latter had replaced the alcohol in the tissues. To the material in acetone cellulose acetate was added daily in increasing amount until the liquid became pasty and viscid. After

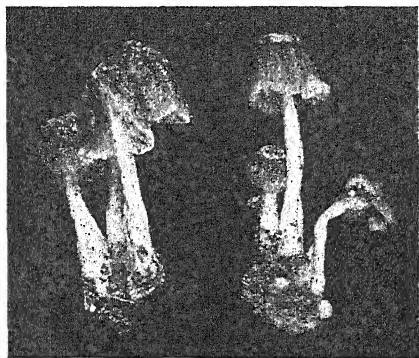


FIG. 3. *Coprinus* impregnated with cellulose acetate.

a fortnight the specimens were removed and the acetone allowed to evaporate, leaving the tissues impregnated with the cellulose acetate. This treatment improves the durability and appearance of the denser fungi greatly as museum specimens (*Polystictus*, *Stereum*, &c.) but it is less effective with the more watery gill fungi. The latter always show a certain amount of shrivelling on drying, and the gills are apt to be drawn together as the cellulose acetate dries, particularly in the case of *Coprinus*, *Agaricus*, *Psalliota*, and *Collybia*. Photographs of dry specimens of *Collybia* impregnated with cellulose acetate are shown in Fig. 2, and of *Coprinus* in Fig. 3.

*Bakelite*. An endeavour was also made to impregnate fungi with bakelite. This substance is produced by the action of a catalyst on a mixture of carbolic acid and formaldehyde and subsequent stoving at over  $100^{\circ}\text{C}$ . It was thought that the tissue of the fungus might act as the catalyst and that the action might possibly proceed partially and slowly at low temperatures within the fungus. Accordingly fungi were soaked in 40 per cent. formaldehyde until fully saturated and liquid carbolic acid allowed to drip over them for some days. On drying the specimens, the tissues were fixed and hardened to some extent, but decreased in bulk and shrivelled more or less, although the gills were fairly well preserved. A specimen of *Pleurotus* preserved in this way is shown in Fig. 4. On stoving such specimens at  $110^{\circ}\text{C}$ . they became too much shrivelled and distorted to be of any value.

*Urotropin*. If ammonia is added to a solution of formaldehyde, a white crystalline solid readily soluble in water, hexamethylene tetramine (urotropin) is formed as an addition compound. Accordingly, fungi soaked

in 40 per cent. formalin were suspended over strong ammonia for a day and then dried. The specimens are rigid and easily handled, but as a con-

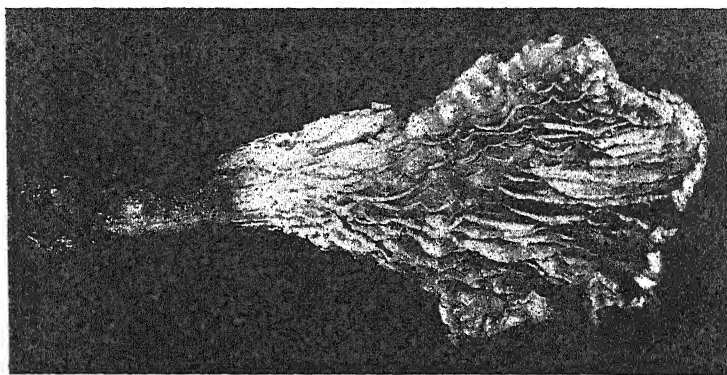


FIG. 4. *Pleurotus* soaked in formalin and carbolic acid and dried.



FIG. 5. *Coprinus* impregnated with urotropin and dried.

siderable amount of water has to be dried out, they become somewhat shrivelled. A specimen of *Coprinus* prepared in this way is shown in Fig. 5.

*Phenolurotropin.* Much more satisfactory specimens were obtained by soaking the specimens in a mixture of two parts of 40 per cent. formaldehyde and one part of liquid phenol, or one part of the liquid obtained by adding just sufficient water to liquefy crystalline carbolic acid. The specimens are dried superficially by blotting paper and then suspended

over strong ammonia. In a few hours, to a day, according to their size, the specimens set quite solid and do not shrivel at all on drying; in addition they lose little weight, their water content having largely and mysteriously disappeared.

The explanation is a fairly simple one. If to the mixture of two parts of formaldehyde and one part of liquid carbolic acid,  $1\frac{2}{3}$  volumes of saturated ammonia are added and the mixture stirred, much heat is produced, and on cooling the liquid sets to a crystalline mass with water suspended in its meshes. This crystalline material is readily soluble in alcohol, ether, chloroform, hot water, and warm benzole; less soluble in cold water, cold benzole and hot petrol ether, and insoluble in cold petrol ether. When heated to over  $70^{\circ}\text{C}$ . it melts and slowly loses water of crystallization. Heated to over  $100^{\circ}\text{C}$ . it gives off ammonia and turns into a yellow quite insoluble substance closely resembling bakelite.

The original crystalline material appears to consist largely of an addition compound of one of urotropin with three of phenol, and when the ammonia is driven off by heat the phenol and formaldehyde remain as the bakelite-like substance. The crystallizing out of the urotropin-phenol compound in the tissues of the fungus removes most of the remaining water as water of crystallization, and at ordinary temperatures this water is permanently retained in solid crystalline form. Hence the absence of shrivelling on drying.

This is by far the most satisfactory method of obtaining permanent dry specimens of delicate fungi without change of shape or structure. They can be handled freely, stored in a small bulk, and used as museum specimens or as class material after soaking out in water. A specimen of *Coprinus* preserved in this way is shown in Fig. 6.

If the soaked specimens are hung up in the ammonia gas by the stipe the weight of the pileus is apt to make it sag to one side. If, however, a thread is passed through the pileus at the top of the stipe and the sporophore suspended in the natural position, the specimen will harden with little or no distortion or alteration of shape. This method of preservation can be used with the most delicate and evanescent fungi.

If specimens impregnated with phenolurotropin are heated to over

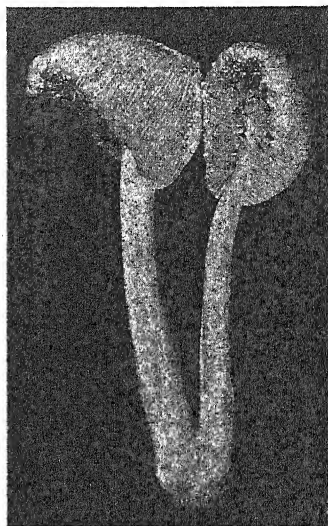


FIG. 6. *Coprinus* impregnated with phenolurotropin.

105° C. they turn yellow or brown as the phenolurotropin<sup>1</sup> is converted into bakelite with loss of water and ammonia. There is, however, no gain in completing the change, since apart from the change of colour, the specimens are more brittle, the impregnating material is now insoluble, and since the phenolurotropin melts at over 70° C. the tissues are apt to undergo some shrinkage or distortion while the water of crystallization is drying out.

One disadvantage of the phenolurotropin impregnated specimens is that if kept in glass-topped boxes, a portion of the phenolurotropin compounds gradually sublimates and appears on the glass. The specimens are, however, unaffected for an indefinite time and they can be kept with perfect safety in open boxes if shielded from dust. They are also not attacked by insects.

#### SUMMARY.

Various modes of preserving fungi are described. By far the most satisfactory method applicable to the most delicate fungi, is to soak in a mixture of two parts of formaldehyde to one of liquid carbolic acid and after superficial drying to suspend the specimen over strong ammonia until it sets solid without drying. The appearance is somewhat like a candied fruit; the shape and structure are fully preserved, and by soaking in spirit or water the whole of the impregnating material can be dissolved away. By heating impregnated specimens to over 100° C. they can be bakelized and are now unaffected by water.

<sup>1</sup> This name is, of course, a popular one only. Shono has investigated in numerous papers (*Japanese Journal of Chemistry, &c.*), the additive compounds by ammonia, formaldehyde, and carbolic acid including 2 : 2' dihydroxydibenzylamine, which appears to be the chief product referred to above as phenolurotropin.



# Studies on the Transport of Carbohydrates in the Cotton Plant.

## III. The Polar Distribution of Sugar in the Foliage Leaf.<sup>1</sup>

BY

E. PHILLIS

AND

T. G. MASON.

With Plate XXIII and thirteen Figures in the Text.

### CONTENTS.

SECTION.	PAGE
1. INTRODUCTION . . . . .	586
2. METHODS . . . . .	587
(a) Preparation and Extraction of Material . . . . .	587
(1) Collection and subdivision of material . . . . .	587
(2) Extraction . . . . .	588
(b) Identification and Estimation of Sugars . . . . .	591
(1) Constituent hexoses . . . . .	591
(2) Free glucose and fructose . . . . .	591
(3) Sucrose . . . . .	593
(4) Polyglucoside . . . . .	594
3. RESPONSE OF LAMINA AND OF PETIOLE . . . . .	600
(a) The Diurnal Effect (Experiments 1 and 2) . . . . .	600
(b) The Effect of Ringing and of Prolonged Darkening (Experiment 3) . . . . .	604
4. CONCENTRATIONS AND CONCENTRATION CHANGES IN MESOPHYLL, VEIN, AND PETIOLE . . . . .	609
(a) Concentrations . . . . .	609
(b) Changes in Concentration (Experiments 1, 2, 4, and 5) . . . . .	610
5. SIEVE-TUBE/COMPANION CELL RELATIONS . . . . .	616
6. VEIN GRADIENTS (Experiment 6) . . . . .	618
7. PHLOEM GRADIENTS (Experiment 5) . . . . .	619
8. REVERSAL OF NORMAL DIRECTION OF TRANSPORT (Experiment 7) . . . . .	624
9. DISCUSSION . . . . .	629
10. SUMMARY . . . . .	631

<sup>1</sup> Paper No. 9 from the Physiological Department of the Cotton Research Station, Trinidad.

## SECTION I. INTRODUCTION.

KRUSEMAN (26), after an exhaustive survey of the literature, has summed up what he considers to be the present state of our knowledge of the problem of transport. 'There is', he says, 'practically unimpeachable evidence that the assimilatory products are transported through the sieve-tubes, but we are as yet entirely in the dark as to the means whereby such high velocities are effected'. The writers are aware of only two attempts to explain the mechanism of this high speed transport through the phloem, namely the *Druckstromhypothese* (37) and its subsequent modifications (7, 8), and the *Diffusion Theory* (10, 15, 33). Some of the difficulties with which they are beset have recently been discussed (34). As the present paper is concerned with the movement of materials out of the leaf rather than with longitudinal transport through the phloem, we will refer only to the difficulties that confront these theories in connexion with the export of materials from the leaf. Schumacher (47) has pointed out that in an isolated leaf there is a rapid passage of nitrogen from the lamina into the veins, and concludes from this 'dass der Wanderprozess wenigstens in seiner Mechanik unabhängig von der Bedarfsfrage ist'. This observation may not be conformable with a simple diffusion plan of transport, but might, as Mason, Maskell, and Phillis (34) have suggested, be explained by the rapid conversion of mobile into non-mobile forms of nitrogen in the vein. We are also indebted to Schumacher (48) for drawing attention to an observation of Mothes (36), who found that there is a considerable export of nitrogen from a wilting leaf. We have here a fact that is difficult to explain in terms of the *Druckstromhypothese*, which would appear to require the maintenance of turgor pressure in the mesophyll.

As we are concerned only with one phase of the problem of transport, and as Mason and Maskell alone appear to have attempted to measure the actual sugar concentrations in the leaf and in the conducting channels, it will be necessary to consider their results in some detail. They found (32, 33) that the concentration of sucrose and of reducing sugars in the mesophyll was in general smaller than in the tissues that serve conduction, i.e. midrib, petiole, and bark of stem; a situation that clearly would not be anticipated on either theory of transport. By means of a new method they were able to form *estimates* of the sugar concentrations in the phloem. These estimates indicated that though the sucrose concentration in the phloem was much greater than in the mesophyll, yet the gradient in reducing sugars from the assimilating cells to the phloem was positive. This led them to suggest that the export of sugar from mesophyll to phloem took place as reducing sugars, and that in the phloem condensation to sucrose occurred. They had to assume that the leaf cells were

relatively impermeable to sucrose, so that leakage back was checked. In this way it was suggested that a considerable head of sugar, mainly sucrose, might be generated in the phloem. Thus they propose a diffusion plan for transport in that actual movement occurs along concentration gradients, even though the total sugar concentration is much greater in the phloem than in the mesophyll. The authors emphasize the tentative nature of the plan proposed and allude to a number of doubtful premises on which it rests. Our object in the present paper is to re-examine the problem, and to test the validity of their theory of the export of carbohydrate from the leaf.

## SECTION 2. METHODS.

### (a) *Preparation and Extraction of Material.*

#### (i) *Collection and subdivision of material.*

Leaves were collected from plants kept in a vegetative condition by periodically removing the flower-buds. Only leaves on the main axis were used, and grading was done on the basis of position on main axis and length of leaf. After collection the leaves were brought into the laboratory, subdivided into lamina and petiole, and weighed. Each sample was then rapidly cut up, mixed, and divided into two portions. One portion was used for moisture determinations, from which the dry weight of the whole sample was calculated. The other was placed in metal tubes and immersed in a freezing mixture at  $-15^{\circ}$  C. preparatory to the expression of the sap for sugar determinations.

In some experiments the larger veins were separated from the rest of the lamina. Where this was done, reference is for convenience made to *vein* and *mesophyll*, even though the latter contains the epidermis and the smaller veins. Where there was no subdivision of the lamina into vein and mesophyll, the term *lamina* is retained.

In some experiments a subdivision of the tissues of the *petiole* was made. The procedure followed very closely the course described by Mason and Maskell (29, 33) for the subdivision of the stem. Thus the bark of the petiole, which includes cortex, rays, fibres, and phloem, was separated from the wood (cf. Pl. XXIII, Fig. 1). The bark in turn was subdivided into two regions. The inner region peeled off readily at the fibres that cap the phloem, and consisted, of course, of rays, phloem, and fibres, while the outer comprised the cortical parenchyma and the epidermis. There was a remarkable constancy in the proportion of these inner and outer regions in any one experiment. Thus in an experiment involving sixteen samples, the fresh weight of the inner region was 13.34 per cent. and the outer 86.66 per cent. of the total, with a standard deviation of 1.01. The amount of inner bark obtainable was of course small and

consequently material could not be spared for determinations of dry weight and total solids. In experiments where subdivision of the petiolar bark was undertaken, sugar concentrations are expressed, therefore, per 100 c.c. sap. In experiments where sap was plentiful and where total solids could be determined the concentrations are expressed per 100 grm. water.

Subdivision of the *bark* of the stem into three regions was also made. In all experiments of this nature, material was preserved for microscopic measurements of the proportion of phloem, rays, and cortex. An estimate of the concentration of sugar in the phloem of the petiole, as well as in the stem, has been made for the first time (cf. 29, 33). In making these calculations the assumption is made that the fibres contribute nothing, and that the sugar concentrations are similar in the parenchyma of ray and cortex.

In experiments involving subdivision of tissues, there is the possibility that the unavoidable handling of the tissues may bring about changes in the sugars present. We compared the sugar concentrations found in the individual tissues with those calculated from the concentrations of the separated fractions, and have not found evidence of such changes (cf. 29). The results of some experiments on the separation of lamina, of petiolar bark, and of stem bark are given in Table I.

TABLE I.

*Comparison of Intact and Subdivided Tissues. Mean Concentrations (grm. per 100 c.c. sap).*

	Lamina.		Petiolar bark.		Stem bark.	
	Subdivided into mesophyll and large veins.		Subdivided into two regions.		Subdivided into three regions.	
	Intact.	Subdivided.	Intact.	Subdivided.	Intact.	Subdivided.
No. of samples	15	15	6	6	6	6
Reducing sugars	0.65	0.63	0.81	0.83	1.22	1.24
Sucrose	0.45	0.47	1.29	1.35	3.34	3.33

## (2) *Extraction.*

(i) *Procedure of Mason and Maskell.* Mason and Maskell (32) packed the material into metal cylinders which were immersed in an ice-salt mixture at approximately  $-16^{\circ}$  C. for about 24 hours. After freezing, the sap was expressed between silver discs in a vice. It was *filtered* and collected in a test-tube jacketed in ice. For the estimation of sugar 5 c.c. of sap were taken and cleared with the minimum of basic lead acetate. The sucrose content of sap obtained from frozen material was compared with that of alcoholic extracts of fresh material. The agreement in the case of bark and wood was found to be satisfactory. In the leaf, however, the freezing method gave somewhat lower values for sucrose than the alcoholic extraction. 'It is quite possible', they say, 'that the sucrose figures found for the leaf may in some cases be considerably below the

real values' (cf. 32). In view of the great importance of obtaining a true estimate of the sucrose content of the leaf, we have re-examined the freezing method.

(ii) *Rate of freezing.* A comparison was made of the sugar content of leaf samples frozen in wide and narrow tubes. The wide tubes were 2 inches and the narrow  $\frac{3}{4}$  inch in diameter. The material was frozen for three hours, and the sap on expression was collected *without filtering* in test-tubes jacketed in a freezing mixture. Three collections of leaves, each of two samples, were compared. The results, which are shown in Table II, do not reveal any difference due to the width of the tubes and consequently to the rate of freezing.

TABLE II.

*Sugar Concentrations (grm. per 100 c.c. sap) after Freezing in Wide and Narrow Tubes.*

		Reducing sugars.				Sucrose.			
		Wide.		Narrow.		Wide.		Narrow.	
Sample.		A.	B.	A.	B.	A.	B.	A.	B.
Collection {	1.	1.13	1.13	1.13	1.12	0.60	0.58	0.60	0.58
	2.	0.92	0.95	0.92	0.95	0.48	0.43	0.46	0.44
	3.	0.63	0.64	0.61	0.63	0.29	0.28	0.30	0.28

(iii) *Time of freezing.* A number of samples of leaves were plunged into a freezing mixture, which was initially at  $-15^{\circ}\text{C}$ . The temperature rose during the course of the first nine hours to  $-5^{\circ}\text{C}$ . The freezing mixture was then re-made and the temperature again lowered to  $-15^{\circ}\text{C}$ . Fifteen hours later the temperature had risen to approximately  $-1^{\circ}\text{C}$ .

TABLE III.

*Sugar Concentrations (grm. per 100 c.c. sap) after Varying Periods in Freezing Mixture.*

Hours.	Reducing sugars.	Sucrose.
1	0.45	0.33
2	0.44	0.33
3	0.44	0.35
4 $\frac{1}{2}$	0.45	0.34
9	0.47	0.31
24	0.64	0.13

It will be seen (Table III) that little inversion occurred up to nine hours, but that there was considerable inversion between nine and twenty-four hours. It seems probable that some of the inversion that occurred in the experiments of Mason and Maskell may have been due to the rise in the temperature of the freezing mixture, for inversion in the leaf can apparently occur at temperatures as low as  $-1^{\circ}\text{C}$ .

(iv) *Pressure.* Leaves were frozen for three hours and pressed in the usual way, but the sap was collected in three separate fractions. The first, of course, required considerably less pressure than the last. The results, which are shown in Table IV, do not indicate any differences between the three fractions.

TABLE IV.

*Concentrations (gram. per 100 c.c. sap) in Successive Fractions.*

	Reducing sugars.	Sucrose.
1st Fraction	0.53	0.35
2nd „	0.52	0.34
3rd „	0.52	0.35

(v) *Comparison of freezing method and other methods of extraction.* As a result of the work just reported, the freezing method now adopted differs slightly from that of Mason and Maskell. The material is frozen in a freezing mixture initially at  $-15^{\circ}\text{C}$ . and left for a period of three hours. The temperature of the mixture is not allowed to rise above  $-5^{\circ}\text{C}$ . Metal containers of not more than two inches diameter are used. The material is pressed in a vice between silver discs, and the sap collected immediately *without filtering* in a test-tube jacketed by a freezing mixture. Clearing is done with a slight excess of normal lead acetate, and deleading with sodium oxalate. The method of calculating the weight of sugar in the sample has previously been described (32).

In order to compare the freezing method of extraction with the boiling water and alcohol methods, six samples of leaves, each of 25 gram., were weighed; two for each method of extraction. The freezing method has just been described. For the alcohol and water methods, three successive extractions were carried out on each sample using 150, 100, and 50 c.c. of boiling solvent respectively. 92 per cent. alcohol was used for the first extraction and 80 per cent. for those succeeding. The combined alcohol extracts were then evaporated at a temperature not exceeding  $40^{\circ}\text{C}$ . and the residue taken up in water. The results are shown in Table V.

TABLE V.

*Sugar Concentrations (gram. per 100 gram. water) by Different Methods of Extraction.*

Method.	Reducing sugars.		Sucrose.		Polyglucoside.	
	A.	B.	A.	B.	A.	B.
Freezing	0.44	0.45	0.39	0.39	0.12	0.17
Alcohol	0.27	0.26	0.41	0.40	0.11	0.14
Water	0.43	0.44	0.34	0.36	0.24	0.30

For the first time we give the results for the polyglucoside fraction.

This fraction represents the difference in the glucose reducing value after invertase inversion and after hydrolysis with hydrochloric acid. As it will be considered later in some detail, it need only be pointed out here that its extraction is much the same for the freezing and for the alcohol methods, but that the water method apparently may cause the solution of some polysaccharide which is not removed by lead acetate.

The agreement between the values for reducing sugars for the freezing and water methods suggests that the alcohol estimate may be low. As we have on a number of occasions found a complete and unaccountable disappearance of sugar when using the alcohol method, sugar destruction may be indicated. The values for sucrose are very similar for the freezing and alcohol methods, and are somewhat low for the water method. To sum up, it appears that the freezing method is preferable to the alcohol (cf. 43) and the water methods of extraction, for there is no loss of sugar such as is liable to occur with the alcohol method, and not the same tendency for the extraction of polysaccharides as with the water method.

(b) *Identification and Estimation of Sugars.*

(1) *Constituent hexoses.*

Tests for the presence of pentoses and pentosans in the cleared sap by the production of furfural have proved uniformly negative. The presence of glucose has been established by the preparation of saccharic acid, which was identified by analysis of its silver salt. The presence of fructose, inferred from the occurrence of an invertase hydrolysable sugar in the sap, was confirmed by colour tests and by measuring the rate of destruction of sugar in the sap with HCl. The sap sugars after preliminary acid hydrolysis were all readily fermented by *S. cerevisiae*, but since this does not exclude the occurrence of d- mannose and d- galactose, these sugars were tested for independently, mannose by the phenylhydrazone reaction and galactose by the mucic acid test, but in neither case could their presence be demonstrated. The hexoses present in the leaf are therefore glucose and fructose.

(2) *Free glucose and fructose.*

(i) *Identification.* The presence of *free* glucose and fructose in plant extracts has been generally assumed from the ready formation of glucosazone, from the reducing power of these extracts, and from fermentation tests. None of these observations serve, however, to differentiate between glucose and fructose. Deleano (14) claimed to have demonstrated the presence of both these sugars in vine leaves by means of the methylphenylhydrazone and the methylphenylosazone respectively, but doubt exists about this demonstration since the possibility of inversion of sucrose

during extraction was not sufficiently guarded against. The polarimeter has been utilized for the determination of glucose and fructose, but so many assumptions (21) are involved in the determination that it is doubtful whether polarimetric observations even establish that both are present. In short, while the presence of either glucose or fructose is beyond doubt, actual demonstration that both are present is lacking, though the simultaneous occurrence of sucrose and invertase might suggest this.

In cotton leaves, we infer the presence of either free glucose or free fructose, or both, from the ready formation of glucosazone, from the reducing power, and from fermentation tests. The presence of free fructose is indicated by the preparation of glucosazone after destruction of the aldose with alkaline iodine. Though no actual demonstration of the presence of free glucose is offered, there can be little doubt that free glucose as well as free fructose is normally present.

(ii) *Estimation.* Glucose and fructose have been estimated jointly by their reducing power, and individually by the destruction of glucose with iodine in alkaline solution. In each case the actual sugar determination was made by the Shaffer Hartman micro-method (50). All solutions were neutralized before the addition of the copper reagent. Invert sugar and glucose were found to possess the same reducing power, and we have therefore assumed that the reducing powers of glucose and fructose are equal (cf. 41). This has made possible the use of glucose calibrators. A set of calibrators was used with each set of determinations, as it was found impossible to standardize conditions so exactly that a single calibration curve could be used, even for each batch of reagents.

The oxidation of aldoses by iodine in alkaline solution has recently been developed into a quantitative method for their determination by Hinton and Macara (19). Our attempts to estimate glucose in cotton leaves by this method have not been successful, the indicated glucose being frequently greater than the total reducing sugars. Determination of the fructose left after destruction of the glucose with iodine, as suggested by Kolthoff (25), has, however, given concordant results. The method<sup>1</sup> consists in the oxidation of aldose in alkaline solution under the exact conditions laid down by Hinton and Macara for the iodimetric estimation of glucose. Excess iodine is then liberated from the alkaline solution by acid and removed by the addition of just enough sodium sulphite. The residual fructose is then estimated by the Shaffer Hartman method. The difference between the reducing power of the solution before and after iodine is taken as a measure of the glucose. To what extent the values found are really due to glucose and fructose will be more conveniently considered in connexion with the polyglucoside.

<sup>1</sup> We are indebted to Dr. E. J. Maskell for his kindness in supplying us with details of the method.



(3) *Sucrose*.

(i) *Identification*. The increase in reducing power that occurs on treatment with invertase is generally accepted as evidence for the presence of sucrose. Kayser (22), in 1883, claimed to have actually isolated sucrose from vine leaves. Schulze and Frankfurt (46), in 1895, in a few instances obtained crystals of sucrose from leaf extracts, but the yield was unexpectedly small, and in most cases their attempts proved abortive, though from other organs no difficulty was encountered in the isolation of sucrose. It is remarkable that since the time of Schulze and Frankfurt, more than thirty years ago, no one, so far as we can ascertain from the literature, has succeeded in isolating sucrose from foliage leaves. A number of workers, Petit, 1873 (40), Brown and Morris, 1893 (5), Parkin, 1912 (39), Gast, 1917 (18), and Kylin, 1918 (27), have, however, considered the agreement between estimates of the sucrose content of the leaf by the copper reduction and optical methods to be close enough to indicate the presence of sucrose. In more recent times the use of the polarimeter for work on the sugar of the leaf has not proved so generally satisfactory, estimates of sucrose by optical and copper methods showing wide divergences [Davis, Daish, and Sawyer, 1916 (12), see also Barton-Wright and Pratt, 1930 (3)]. Very recently, though, Schroeder and Herrmann (44), while working on the effect of water content on the sugars of the leaf, have obtained good agreement between the two methods.

In extracts from the vegetative tissues of the *cotton plant* invertase brings about an increase in reducing power. The increase in reducing power is, moreover, due equally to glucose and fructose. The rate of hydrolysis by invertase of leaf and stem tissue extracts is similar to that of pure sucrose under the same conditions. On the other hand, comparison of the rates of hydrolysis by acid of the apparent sucrose of the leaf and of pure sucrose added to leaf extracts has given, thus far, discordant results. The hydrolysis was brought about with N. HCl at 27° C. Further, our attempts to isolate sucrose by the methods of Schulze and Frankfurt (46) and of Winterstein (54) from cotton leaves have not been successful, and estimates of the sucrose content of the leaf by the copper and optical methods have differed widely. For the present, however, we will assume that the invertase hydrolysable fraction of the leaf, as well as of other vegetative tissues, is sucrose. Raffinose, of course, is present only in the seed.

(ii) *Estimation*. Sucrose has been estimated by the increase in reducing power brought about on invertase inversion. Tests with known amounts of sucrose added to saps showed this procedure to be justifiable (cf. 41). Sucrose values throughout this paper are expressed as the corresponding invert sugar values.

(4) *Polyglucoside.*

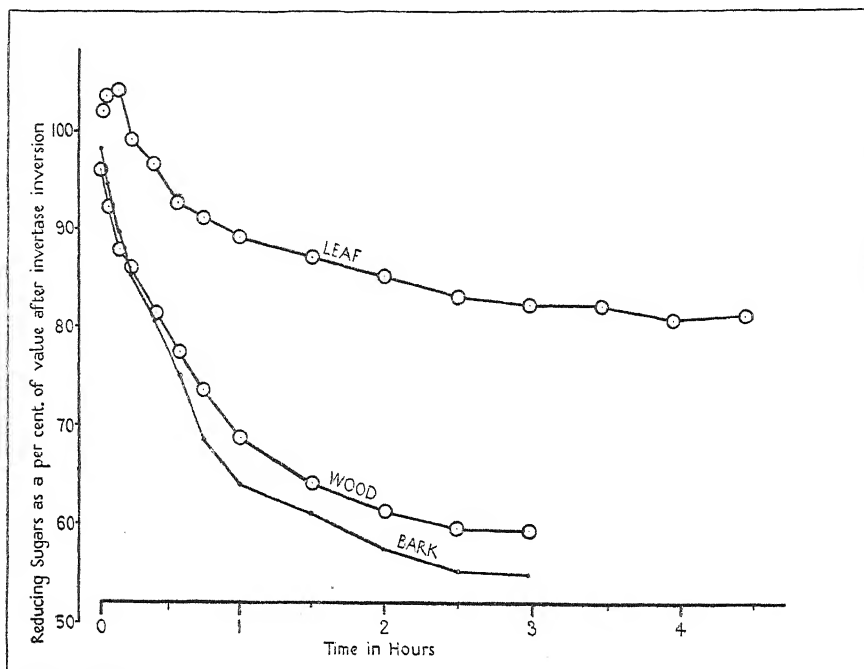
(i) *Identification.* On boiling leaf extracts with approximately 0.6 N. HCl Brown and Morris (5) found that the increase in reducing power exceeded that due to the action of invertase. They claimed that the excess brought about by acid hydrolysis was due to maltose, and based their claim on the preparation and isolation of maltosazone and on the increase in reducing power brought about by the enzyme maltase. Kluyver (24) and Gast (18), using selected strains of yeast, also claimed to have demonstrated the presence of maltose in foliage leaves. Their findings, as are those of Brown and Morris, are, however, somewhat doubtful in view of the methods of extraction adopted. Keulemans (23) also thought that he had demonstrated the presence of maltose. As he extracted with boiling alcohol, and also used selected strains of yeast, his claim merits consideration. Onslow (38), however, in commenting on his work, remarks 'the impression is given that considerable errors may have been introduced owing to the complexity of the method, and the introduction of yeast preparations into the sugar extracts'. In fact, the work of Davis and his collaborators (12, 13), whose method involved alcohol extraction of the fresh material and differential fermentation for the determination of maltose, renders it very improbable that maltose is normally present in the foliage leaf.

Though maltose may be absent from the green leaf, it is certain that an increase in the reducing power, in excess of that due to the inversion of sucrose, is very commonly brought about by boiling leaf extracts with acid under conditions approximating to those of Brown and Morris [cf. Campbell (6), Kylin (27), Schroeder and Horn (45), Horn (20), Ahrns (1), Bruns (4)]. Most of those who have used this method have assumed the presence of maltose. It is remarkable that in no instance has an allowance been made for the destruction of fructose. If this correction is made for certain of the results of Brown and Morris, the initial reducing power of the maltose, thus calculated, is greater than that actually found. It would appear, in short, that while the presence of a polyglucoside<sup>1</sup> of unknown constitution is of common occurrence in foliage leaves, its identity with maltose is very doubtful.

Mason and Maskell (33) found evidence of a polyglucoside in the leaves of the cotton plant. In the stem tissues, however, they were doubtful of its presence. It occurred to us that it might be instructive to compare the hydrolysis curves of extracts from leaf, bark, and wood. The hydrolysis was brought about by boiling N. HCl. The results, which are expressed as percentages of the values after invertase inversion, are shown

<sup>1</sup> While the term *polyglucoside* will be used in referring to this fraction, it must be emphasized that it is merely used for convenience in distinguishing it from the usual polysaccharide (2) fraction. Moreover, it is uncertain whether some fructose may not also be present.

in Text-fig. 1. It will be observed that the curves differ in that, while the reducing power falls immediately for bark and wood, it increases for about ten minutes in the case of the leaf and then decreases. The curves for

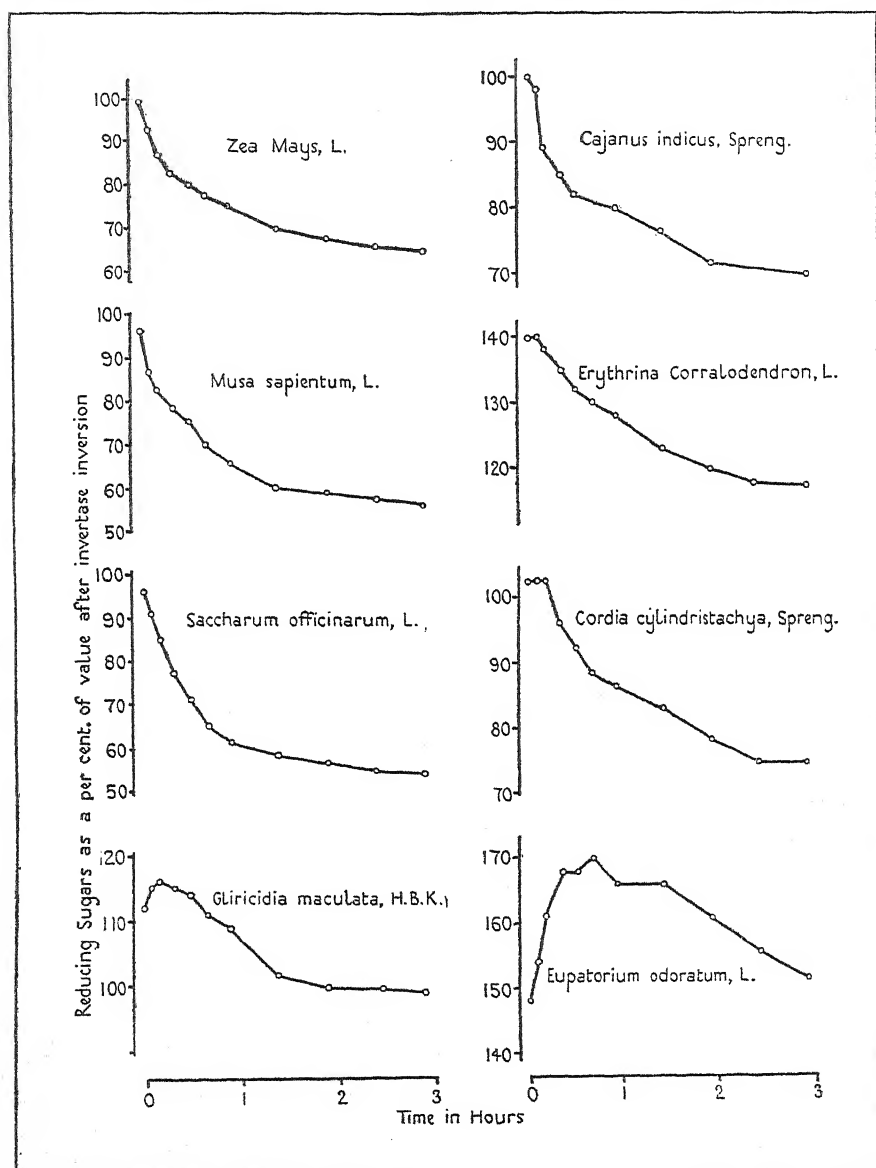


TEXT-FIG. 1. Hydrolysis curves of leaf, bark, and wood extracts.

bark and wood are similar to that for sucrose and register the destruction of fructose; the difference in the level finally obtained being due to differences in the ratio of total glucose to total fructose. For the leaf the curve rises above the invertase level, but the difference between the latter and the maximum attained is of course an underestimate of the polyglucoside by an amount equal to that of the fructose destroyed during the first ten minutes of the hydrolysis. The time at which the maximum is attained varies considerably in different samples, and depends on the ratio of polyglucoside to total fructose. It seems clear from the curves that a polyglucoside is present in the leaf, and that it is either absent or only present in very small amounts in the tissues of the stem.

At this stage we may digress to consider the hydrolysis curves of a number of plants other than cotton. The leaves of three mono- and five dicotyledons have been examined. The results are shown in Text-fig. 2, and are again expressed as percentages of the total sugar after inversion by invertase. It will be seen that the relative amount of polyglucoside varies considerably. In *Eupatorium odoratum* the amount present must be almost

equal to that of the total sugars after invertase inversion. It is a strange coincidence that all the monocotyledons examined tend towards the



TEXT-FIG. 2. Hydrolysis curves of leaf extracts of three mono-, and five dicotyledons.

sucrose type of curve, while the dicotyledons have curves indicative of the presence of a polyglucoside. Kylin (27), it should be pointed out, has recorded the presence of polyglucosides in a number of monocotyledons.

It was thought that the presence of the polyglucoside might be associated with the presence of starch. Tests for starch ruled out this possibility, for all the leaves examined, with the exception of those of the sugar cane, contained starch.

Reverting to the results shown in Text-fig. 1, it seems probable that the initial rate of hydrolysis of the polyglucoside of the cotton leaf is much greater than that of maltose. The rate of hydrolysis of the polyglucoside is, however, best examined by using leaves kept in the dark for five or six days, for under these conditions only traces of sucrose remain, while the amount of the polyglucoside does not change appreciably. In Text-fig. 3 are shown the rates of hydrolysis of the polyglucoside in sap expressed from darkened leaves, and of maltose. The rate of fructose destruction in sucrose is also recorded. In this case the rate of hydrolysis is very rapid, and destruction of fructose appears to begin immediately. In each case the results are expressed as a per cent. of the maximum reducing sugar value. It will be observed that the rate of hydrolysis of the polyglucoside is very much more rapid than that of maltose, and is also more rapid than the rate of destruction of fructose.

One other fact of interest emerges from this graph. The initial reducing powers of both maltose and the polyglucoside are about half the maximum reducing power. This tendency for extracts from darkened leaves to double their reducing power on hydrolysis is shown in Table VI. In extracts prepared from leaves after prolonged (about six days) darkening, not only is the reducing power increased by about 90 per cent., but thus far we have completely failed to obtain a glucosazone or any other form of osazone from them. It would appear that the initial reduction cannot be due to free hexoses. The results suggest that the polyglucoside has initial reducing power.

TABLE VI.

*Per cent. Increase in Reducing Power on Acid Hydrolysis of Sugar  
Extracts from Darkened Leaves.*

Date.	Per cent. increases.
February 19, 1931	84
March 26, 1931	87
April 4, 1931	92
May 5, 1931	78
September 1, 1931	101
November 20, 1931	89
January 1, 1932	100
March 2, 1932	100
	<hr/> 91.4

We have not yet found an enzyme that attacks the polyglucoside; sap expressed from fresh leaves, as well as preparations of emulsin and

maltase being without effect. Fermentation tests have yielded thus far indecisive results. For the present what appears to be important is that the cotton leaf contains a polyglucoside that is soluble in water and 80 per cent. alcohol, and that this polyglucoside is either absent from or is present in negligible amounts in bark and wood. Moreover, it seems probable that it has initial reducing power, and consequently that our *estimates of the hexoses, glucose, and fructose are maximal ones*. Furthermore, we are uncertain to what extent the initial reduction of the polyglucoside, if such really exists, is due to aldose and ketose groups.

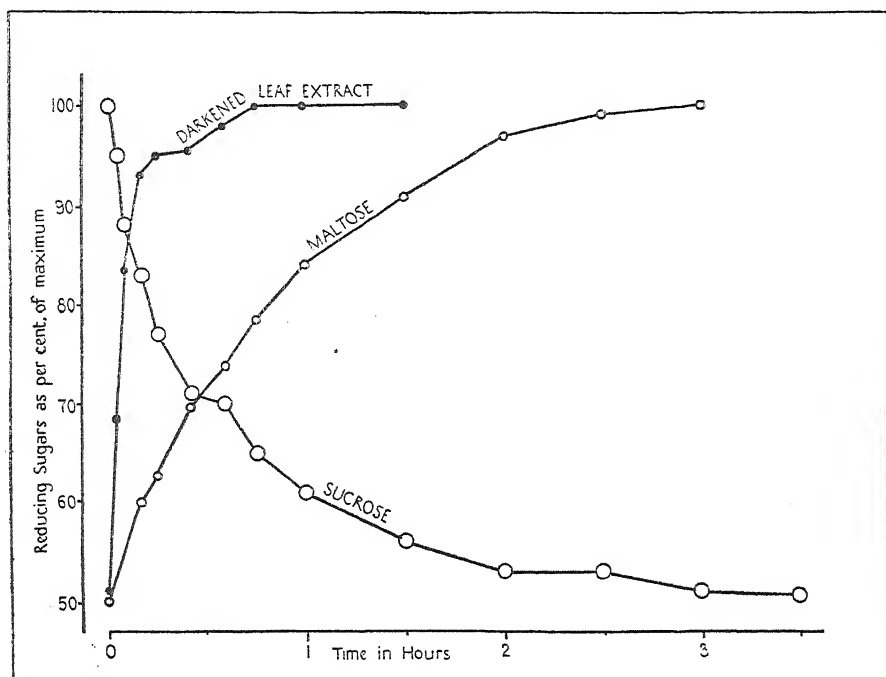
(ii) *Estimation*. The estimation of the polyglucoside has presented some difficulty. No enzyme has yet been obtained which has any action on it. Mild hydrolysing agents such as are employed to invert sucrose are without effect. Consequently the hydrolytic agent employed must be of such a strength that fructose is destroyed. The increase in reducing power over that due to invertase inversion is therefore not a true measure, for it is an underestimate by the extent to which fructose is destroyed. The polyglucoside requires about an hour's boiling with N. HCl to bring about complete hydrolysis, and in that time about 80 per cent. of the total fructose is destroyed.

As glucose and fructose are the only hexoses present in the cotton plant, and as they are normally present in not greatly dissimilar proportions, we have used invert sugar to study the effect of acid on fructose destruction (11). Text-fig. 3 shows the rate of fructose destruction on boiling invert sugar with N. HCl. Destruction is initially rapid, and appears to be complete in three hours. At the end of this period almost 50 per cent. of the total sugar had been destroyed. The loss is presumably due to fructose, for little or no further destruction took place on increasing the time of hydrolysis. The sugar left after three hours has therefore been taken as a measure of the total glucose initially present in the extract. It is, of course, possible that the sugar left after three hours is not entirely glucose, for there may have been some destruction which was balanced by undestroyed fructose.

The action of acids on the hexoses has been the subject of much discussion. There is general agreement that fructose is destroyed by acid, but disagreement as to the extent to which it is destroyed under any given set of conditions. There is also disagreement about the extent to which glucose is destroyed. A method of fructose estimation involving acid destruction was introduced by Sieben in 1884 (49); fructose was completely destroyed by boiling for three hours with 2.25 N. HCl, while glucose was only affected to the extent of 1.5 per cent.. Mason and Maskell (33) found approximately 60 per cent. destruction of fructose, and no destruction of glucose on boiling for three hours with 0.6 N. HCl.

This method of determining total glucose was also tested on sucrose

added to tissue extracts, for it was suspected that fructose destruction might be slower in the tissue extract than in aqueous solution, owing to the buffering effect of the salts present. The sap was first boiled for three



TEXT-FIG. 3. Hydrolysis curves of sucrose, maltose, and of darkened leaf extract.

hours with N. HCl to destroy the fructose. Sucrose was then added to one-half, the other half being used as a control. It was found that 50 per cent. of the sucrose was again destroyed in three hours. Destruction of the sap sugars proceeded very slowly after the first three hours. In the second three-hour period the loss was less than 2 per cent.

The estimation of the polyglucoside therefore requires, firstly, the determination of the sugar left after three hours' boiling with N. HCl. This is the total glucose present in the extract. Secondly, it requires the determination of the total glucose after invertase inversion (this is determined indirectly by the iodine method). The difference represents the glucose liberated by hydrolysis of the polysaccharide and has been used as a measure of the polyglucoside.

The determinations made in a full analysis of the sugars were as follows:

- (1) Total initial reducing power.
- (2) Initial reducing power due to ketoses.
- (3) Total reducing power after invertase inversion.

- (4) Reducing power after invertase inversion due to ketoses.
- (5) Reducing power after three hours' boiling with N. HCl.

These determinations give the following fractions:

- (1) Glucose.
- (2) Fructose.
- (3) Hexoses.
- (4) Sucrose.
- (5) Polyglucoside.

It should be added that in tissue extracts from bark and wood, which do not contain the polyglucoside, the total sugars can be determined by means of the total reduction after invertase inversion, and also from the sum of the sugar left after three hours' boiling with HCl and the fructose after invertase inversion, and that the results given by the two methods do not differ appreciably. In one experiment, in which twenty-four samples of bark were analysed, the mean total sugar concentration after invertase inversion was 4.65 grm. per 100 c.c. sap, while the mean total sugar concentration determined from the sum of the total glucose and total fructose was 4.68 grm. per 100 c.c. sap.

Bark and wood extracts provide data from which two independent values for total fructose can be obtained, the one by the fall in reducing power on acid hydrolysis, and the other by direct determination of the fructose in inverted extracts after the oxidation of glucose by iodine. We have found good agreement between the two values. Thus in the experiment just mentioned, the mean fructose value as determined by acid hydrolysis was 2.09 grm. per 100 c.c. sap, while the mean of the direct determinations was 2.14 grm. per 100 c.c. sap. We may infer from this that the whole of the reducing substance left after oxidation with iodine is definitely fructose.

### SECTION 3. RESPONSE OF LAMINA AND OF PETIOLE.

#### (a) *The Diurnal Effect (Experiments 1 and 2).*

Work on the sugars of the foliage leaf has for the most part aimed at determining the sequence of changes that occur during photosynthesis. In general it has been found that the major response to the diurnal cycle is in sucrose (cf. 38). The work of Mason and Maskell, as we have already noted, suggested that in the leaf of the cotton plant reducing sugars fluctuated to a greater extent than sucrose. As there are grounds for thinking that inversion of sucrose occurred during the preparation of their leaf extracts, we have re-investigated the question of the diurnal changes in the sugar content of the leaf. Throughout this section the results are expressed on the sample basis.



The importance assigned to diurnal changes in the sugars of the leaf for the problem of transport will depend on the nature of the mechanism contemplated for movement through the plant. Mason and Maskell (32) found that the passage of sugar from leaf to boll is much more rapid by day than by night. Thus, if sugar transport conforms to a *Diffusion* plan, the cause of the diurnal change in the rate of transport might be sought in the diurnal change in the sugar concentration of the leaf, and it would be reasonable to assume that the sugar that fluctuates most markedly is, if not actually the most important form in which carbohydrates are exported from the leaf, at least mainly responsible for the diurnal changes in the rate of transport. It will be clear that diurnal changes in the rate of utilization or of ease of transport (cf. 30), if such occurred, would also result in diurnal changes in the rate of transport.

It is difficult to predict what would occur on the *Druckstromhypothese*. On the one hand, the rate of movement of the 'druckstrom' might be less by day than by night, for the turgor pressure of the leaf is generally smaller by day. On the other hand, the actual concentration of sugar in the 'druckstrom' would be smaller during the night. It is conceivable that the rate of transport might even be more rapid by night than by day as Tschesnokov and Bazyrina (52) assert. In plants where transport is more rapid by day than by night, however, as it is in cotton, the sugar that fluctuates most must also be the sugar mainly responsible for the diurnal changes in the rate of transport.

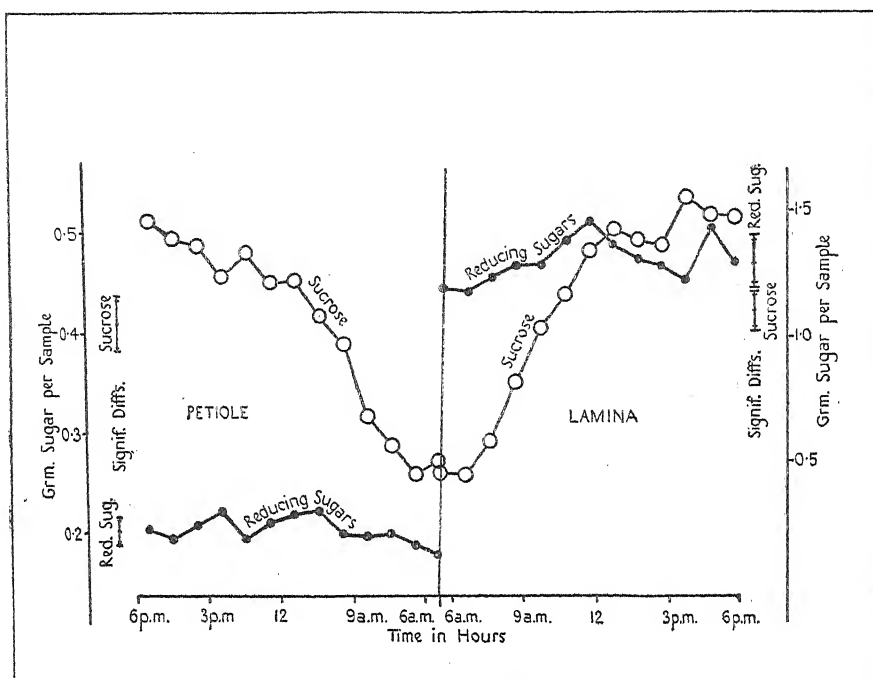
Very little work seems to have been done on the export of sugar from the leaf. Strakosch (51) thought the export from the mesophyll to the vein took place in the form of hexoses and that condensation to sucrose took place on arrival in the vein, a view substantially the same as that of Mason and Maskell. Davis and his collaborators, on the other hand, considered that movement from the mesophyll occurred as sucrose and that inversion occurred during transport along the veins and petiole. Both views involve chemical transformation, but in neither case was any particular mechanism of the movement envisaged.

#### *Experiment 1. April 18th, 1931.*

*Procedure.* Leaves were collected at hourly intervals between 5.30 a.m. and 5.30 p.m., that is to say, between dawn and dusk. Only sucrose and reducing sugars were determined, the primary object of the experiment being to ascertain the extent of the diurnal change in each. In the laboratory the leaves were separated into lamina and petiole. The results are expressed on the sample basis and represent the actual weight of sugar in the sample. There were two samples per collection and 100 leaves per sample.

*Results.* The hourly variations in sucrose and in reducing sugars are

shown in Text-fig. 4. Unlike the results of Mason and Maskell, the change in the total sugars of the lamina is almost entirely due to sucrose. The diurnal changes in the sugars of the cotton leaf thus fall into line with



TEXT-FIG. 4. Hourly variations in sugars (gram. per 100 leaves) of lamina and petiole (Experiment 1).

those of most plants that have been investigated. The transport significance of this observation lies in the suggestion it contains that sucrose, and not the reducing sugars, is responsible for the diurnal changes in the rate of transport to which we have just referred. It does not of course follow that sucrose is the only form in which carbohydrate travels from the mesophyll to the vein, for reducing sugars and the polyglucoside might also play some part in export.

Mason and Maskell found that the diurnal change in the sugars of the bark was mainly due to sucrose. It will be seen (Text-fig. 4) that this is also true for the petiole. Thus the diurnal changes in the sugars of leaf, petiole, and bark all appear to be mainly due to sucrose. The difference in the proportion of reducing sugars in lamina and petiole is very marked, the proportion in the former being very much greater than in the latter.

It is of interest to note that the sucrose of the lamina increased in amount during the day by 2.5 times its initial value, while in the petiole the increase was 0.9 times the initial value. As probably more than 75 per

cent. of the cells of the lamina contain chlorophyll, and are therefore presumably engaged in the manufacture of sugar, the extent of the increase in the leaf is not surprising, but that the whole petiole (cf. Plate XXIII, Fig. 1), which has less than 4 per cent. of phloem, should nearly double its sucrose content is somewhat unexpected. In the case of the stem bark in the experiments of Mason and Maskell the diurnal change in the sucrose content was relatively small, and it was found that the greater part of this change could be attributed to variations in the phloem. It would appear from the degree of response in the petiole to changes in the lamina, either that escape of sucrose from the phloem takes place with great rapidity, or that the cortical parenchyma is responsible for the transport of a certain amount of the carbohydrate exported from the leaf. The matter will be considered further in the next section, which is concerned with sugar concentrations and with subdivision of the petiole. The rapidity with which the petiole responds to changes in the lamina is noteworthy.

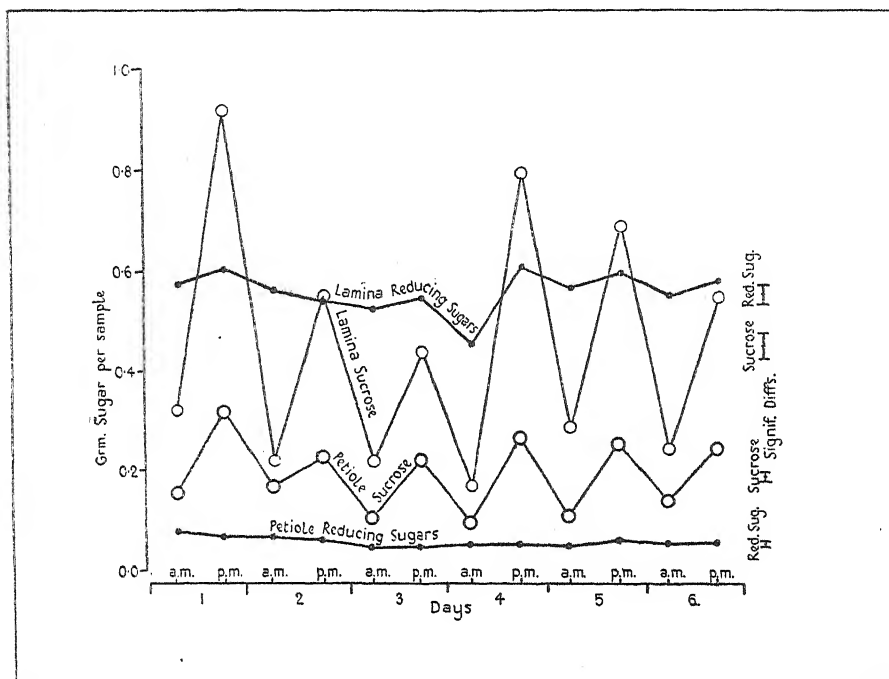
*Experiment 2. October 5th, 1931.*

As the experiment just reported was made at the height of the dry season, and as the ratio of sucrose to reducing sugars can be affected by changes in aridity (1, 4, 20), it was judged desirable to determine the diurnal changes in the sugars of the leaf under more humid conditions than those of the last experiment. It is true that the change in ratio of sucrose to reducing sugars that occurs on desiccation appears to be due to the conversion of starch to sucrose rather than to any direct effect of desiccation on the proportion of sucrose and reducing sugars, but the possibility nevertheless presented itself that reducing sugars might fluctuate more markedly during the day under more humid conditions. The experiment now reported was therefore made during the rainy season.

*Procedure.* The experiment differed from the previous one in that, instead of making collections at hourly intervals during a single day, leaves were collected twice a day over a period of six days. One collection was made at dawn each day, when the amount of sugar is nearly at a minimum, and the other at dusk. There were two samples per collection and each sample contained forty leaves. The results are again expressed on the sample basis.

*Results.* The daily changes in the sugars of the lamina and petiole are shown in Text-fig. 5. The results are similar to those of the previous experiment. In the lamina sucrose fluctuates markedly, while the changes in reducing sugars are small. In the petiole the sequence of changes is essentially the same, except that there is no indication of any diurnal change whatever in reducing sugars. Heavy rain fell about midday on the second and third days of the experiment, and this is reflected in low values for sucrose at the evening collections on those days.

These experiments, the one recording changes through a day and the other the approximate daily maxima and minima for a week, make it clear that in lamina and petiole the diurnal change in sugars is almost entirely



TEXT-FIG. 5. Daily variations in sugars (gram. per 40 leaves) of lamina and petiole (Experiment 2).

due to sucrose, and further that this obtains irrespective of the humidity of the environment. It may be that the consumption of water in the process of sugar synthesis during illumination leads to local desiccation of the chloroplast, and that this desiccation leads to the production of sucrose rather than reducing sugars, in the same way that the conversion of starch to sucrose in darkened leaves is hastened by desiccation. However this may be, the results point to sucrose as the most important form in which carbohydrates travel, both in lamina and in petiole.

(b) *The Effect of Ringing and of Prolonged Darkening.*

If sucrose is the head for carbohydrate transport in the leaf, a block in the channel of transport might be expected to lead to its accumulation. To block the channel of transport, we ringed the stem at ground level. It will be clear that even if the operation of ringing were to bring about an initial accumulation of sucrose rather than of reducing sugars in the lamina, it would not follow that sucrose is of necessity the mobile sugar in the

mesophyll, but it would be the most direct explanation of its accumulation. On the other hand, were ringing to bring about an initial response of reducing sugars rather than sucrose, it would cast doubt on the suggested mobility of sucrose from mesophyll to vein.

*Experiment 3. November 20th, 1931.*

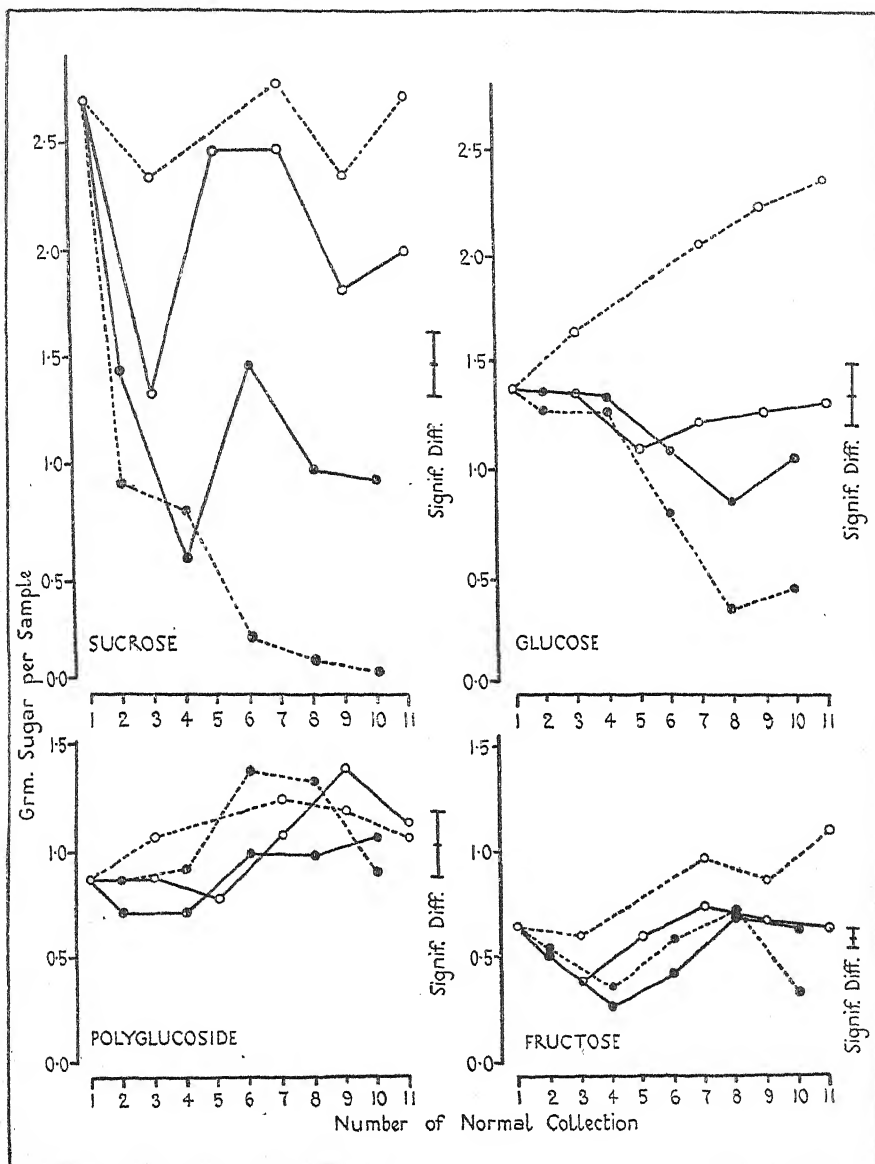
*Procedure.* In the present experiment all the sugar fractions were determined. Collections of leaves from normal plants were made in the early morning and in the afternoon. In addition there were collections of leaves from ringed plants in the afternoon, and of darkened leaves in the early morning. The latter were collected just before the first normal collection and were placed in a dark room with their petioles dipping into water, which was changed daily. The time-table below shows the sequence of operations.

*Time-table.*

November 20.	1.0 p.m.	Plants of ringed group ringed at ground-level.
"	2.0 p.m.	Leaves of dark group collected and placed in dark room.
"	3.0 p.m.	1st collection: normal leaves.
" 21.	6.30 a.m.	2nd collection: normal and darkened leaves.
"	4.0 p.m.	3rd collection: normal and ringed leaves.
" 22.	6.30 a.m.	4th collection: normal and darkened leaves.
"	4.0 p.m.	5th collection: normal leaves.
" 23-25.		6th-11th collections: as on 21st.

There were two samples per collection and fifty leaves per sample. The results are expressed on the sample basis. Lamina and petiole were, as usual, separated.

*Results.* The results for the lamina are shown in Text-fig. 6. There are four curves for each of the fractions determined, viz. normal morning, normal afternoon, ringed, and darkened. We will consider first the diurnal responses, which are of course shown by the differences between the morning and the afternoon values. Sucrose as usual shows well-defined daily fluctuations. On the second day the evening value is less than the morning value, but heavy rain fell during the afternoon. The two hexoses, glucose and fructose, exhibit no consistent difference between the morning and the afternoon. It is interesting to observe that there appears to be about twice as much free glucose as fructose in the lamina. For the polyglucoside, diurnal results for which are presented for the first time, the afternoon values are on the average slightly in excess of the morning, but the mean difference is only of the order of the standard deviation due to sampling. The amount of polyglucoside in the leaf appears to be intermediate between the amounts of glucose and fructose. It must, however, be recollected that the hexose values are maximal ones, as the polyglucoside may have free reducing groups.



TEXT-FIG. 6. Effect of ringing and darkening on sugars (gm. per 50 leaves) of lamina (Experiment 3).

- Normal morning.
- Normal afternoon.
- Darkened.
- Ringed.

In considering the effect of ringing it will be convenient to compare the ringed group with the afternoon values of the normal group, for both were collected at approximately the same times. Sucrose, glucose, and fructose all show marked responses. For the polyglucoside the ringed group at first exceeds the normal afternoon, but the latter finally increases and overtakes it. The effect of ringing on the polyglucoside is thus doubtful, but the absence of a diurnal response in any case renders it unlikely that it is of importance in movement out of the lamina.

The response of sucrose and of the two hexoses requires further examination. Possibly the best way of showing the differences in their behaviour is to consider the responses at the time of the third normal collection, that is to say, after the plants had been ringed approximately twenty-four hours, and at the time of the last collection. If we take the differences between the ringed and the normal afternoon values and express the differences as percentages of the normal values, we obtain the figures shown in Table VII.

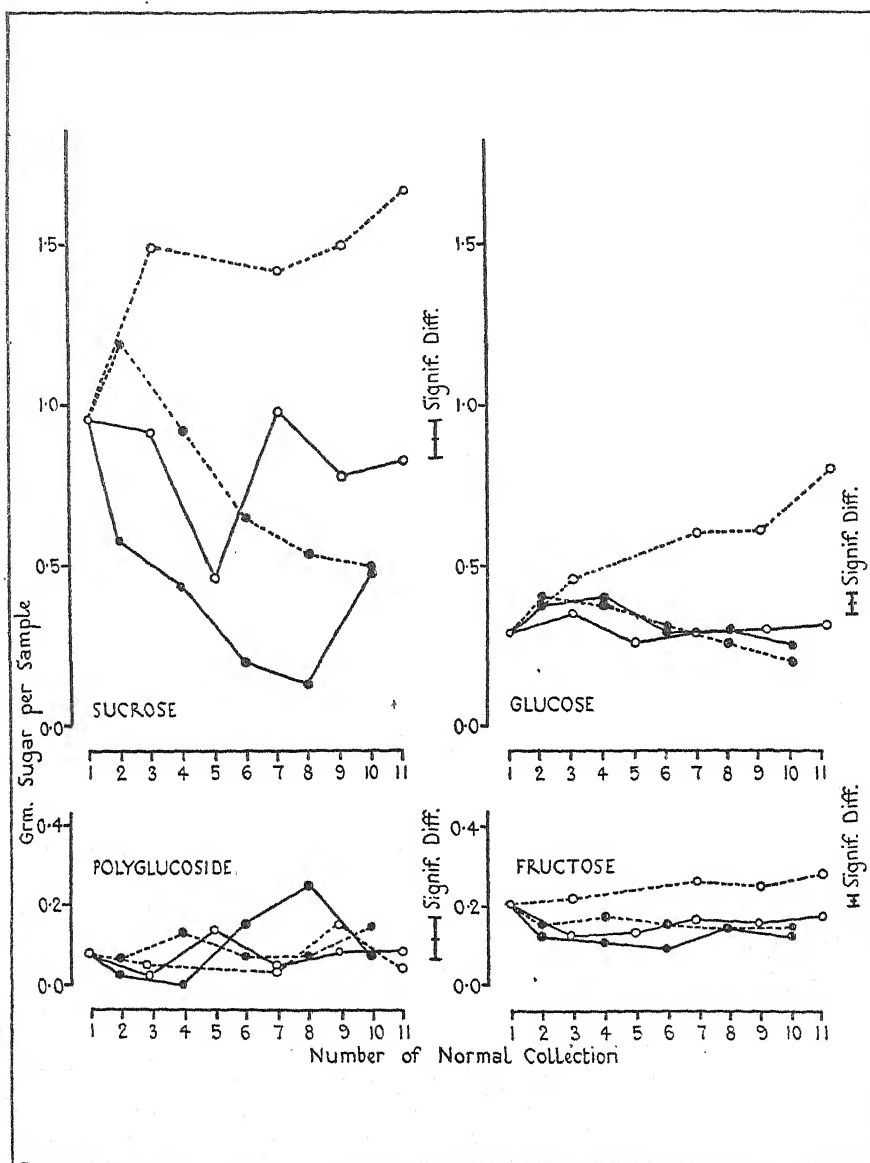
TABLE VII.

*Percentage Increase of Ringed over Normal Values at Third and at Eleventh Collections.*

Collection	No. 3.	No. 11.
Sucrose	76	34
Glucose	22	81
Fructose	62	72

The responses of the three sugars, as measured in this way, show striking differences. The sucrose response is initially very great and then declines, while the response of glucose, and to a lesser extent fructose, show the reverse behaviour. The great initial response of sucrose is quite in accordance with the view that it is the main sugar of export from the lamina.

The results for the darkened and for the normal morning series are not comparable, for in the former both sugar synthesis and export out of the petiole were stopped, while in the latter, of course, sugar synthesis proceeded by day and export of sugar from the petiole took place throughout the whole period. The very rapid initial loss of sucrose from the darkened group once more suggests that it is the mobile sugar. The loss of glucose is at first much slower. The behaviours of fructose and the polyglucoside are ambiguous, but they appear to be much less affected than either sucrose or glucose. After prolonged darkening a little fructose is always found, and as sap prepared from darkened leaves does not yield glucosazone, it seems unlikely that we are dealing with free fructose. The fate of fructose and of the polyglucoside in darkened leaves requires further investigation.



TEXT-FIG. 7. Effect of ringing and darkening on sugars (gm. per 50 leaves) of petiole (Experiment 3).

- Normal morning.
- Normal afternoon.
- - - ● Darkened.
- - - ○ Ringed.

In the petiole (Text-fig. 7) there is as usual a marked diurnal response in sucrose. For fructose the afternoon values are also somewhat greater



than the morning values, but for glucose the reverse obtains until the sixth collection. The amount of polyglucoside is very small and no consistent diurnal effect is evident. The response to ringing is very similar to that of the leaf, for sucrose, glucose, and fructose are all affected; the sucrose response being initially very large and quite unlike the slow increases in glucose and fructose. Ringing does not apparently affect the polyglucoside. The results as a whole suggest that sucrose is the mobile sugar in the petiole.

The results for the darkened series are interesting in that sucrose shows initially an actual increase presumably because, while export from the leaf continued, export from the petiole ceased. It will be noticed that the values for the darkened series exceed those of the normal morning series.

Summarizing the results of the experiments presented in this section, it would appear that sucrose is the most important form in which carbohydrates travel from the mesophyll into the veins as well as longitudinally down the petiole. The response to diurnal changes is almost entirely due to sucrose. Sucrose also shows a rapid response to ringing and disappears with great rapidity from the lamina on darkening.

#### SECTION 4. CONCENTRATIONS AND CONCENTRATION CHANGES IN MESOPHYLL, VEIN, AND PETIOLE.

##### (a) Concentrations.

Reference has already been made to the observations of Mason and Maskell (32, 33) on the sugar concentrations in the mesophyll and in the tissues that serve conduction. The lamina, after removal of the midrib, they termed the leaf parenchyma (cf. 29); it is identical with our mesophyll, and consists of the mesophyll with a small proportion of vein and of epidermal cells. We reproduce (Table VIII) the concentrations of sucrose and reducing sugars found by them in the various parts of the leaf at mid-day, while transport presumably was active.

TABLE VIII.

*Concentrations in Leaf-parenchyma, Leaf-midrib, and Petiole.*

	Parenchyma.	Midrib.	Petiole.
Sucrose	0.345	0.877	0.879
Reducing sugars	1.272	1.632	1.118

The sucrose concentrations in midrib and in petiole are more than twice as great as that in the leaf-parenchyma; a condition difficult to harmonize with a *diffusion theory* of transport with sucrose as the mobile sugar. Yet the experiments in the previous section have all pointed to sucrose as being

the mobile sugar! The concentration of reducing sugars is also greater in the midrib than in the leaf-parenchyma, while in the petiole and the leaf-parenchyma it is of the same order.

We have re-examined the concentrations in the various parts of the leaf and have included all the fractions in our analysis. The main veins were removed from the lamina, and the petiole was subdivided into bark and wood. The results are shown in Table IX, and accord with those of Maskell and Mason (29). The concentration of sucrose is much smaller in the mesophyll than in any of the other tissues. The glucose concentration is also smaller than in the vein, while that of fructose is nearly the same in mesophyll and vein. The polyglucoside alone shows a well-defined positive gradient. That it is the mobile sugar seems unlikely, for it shows little or no response to ringing, and does not disappear after prolonged darkening. Moreover, the diurnal change in the rate of transport is unaccompanied by any marked diurnal change in its concentration.

TABLE IX.

*Concentrations (grm. per 100 c.c. sap) in Various Parts of Leaf.*

	Lamina.		Petiole.	
	Mesophyll.	Vein.	Bark.	Wood.
Sucrose	0.61	1.04	1.06	1.13
Glucose	0.41	1.15	0.42	0.71
Fructose	0.23	0.20	0.09	0.29
Polyglucoside	0.29	0.02	0.05	0.01

(b) *Changes in Concentration (Experiments 1, 2, 4, and 5).*

In the last section we were concerned primarily with the fluctuations in the amounts of the sugars within the various tissues of the leaf. The results were expressed on the sample basis, and therefore were independent of moisture changes in the tissues. The object was to determine the most probable form in which carbohydrates are exported from the leaf.

In the first part of the present section the sugar concentrations in the various tissues have been compared. It was shown that sucrose, which the experiments recorded in Section 3 indicated as the sugar of transport, was present in much greater concentration in petiole and in vein than in the mesophyll.

Thus sucrose is either accumulated by the vein against a gradient, or else there is a static component of some sort in the vein. This static component might be regional, that is to say, the gradient from mesophyll to phloem might be positive and transport might take place across a sheath region with a higher mean concentration of sucrose than either mesophyll or phloem.

A further possibility is that sucrose occurs in some loose form of com-

bination in the vein. In this case accumulation in the vein would be due to a mechanism essentially similar to that suggested by Mason and Maskell, whereby hexoses are condensed to sucrose. We have no evidence that there is any difference, physical or chemical, between the sucrose of the mesophyll and that of the conducting tracts, although such differences may exist and are removed by the methods of analysis employed. We shall not, therefore, consider this possibility in subsequent discussion.

It will be clear that if the diurnal *fluctuations* in sucrose concentration in vein and petiole were to exceed those in the mesophyll, the presence of a static component would not be a sufficient explanation of the negative gradient, and it would seem reasonable to infer the presence of a negative dynamic gradient in sucrose. Similarly, if transport were checked by ringing the stem and this led to a greater accumulation of sucrose in the conducting tracts than in the mesophyll, the accumulation of sucrose against a gradient by the vein, or some part of it, would be indicated.

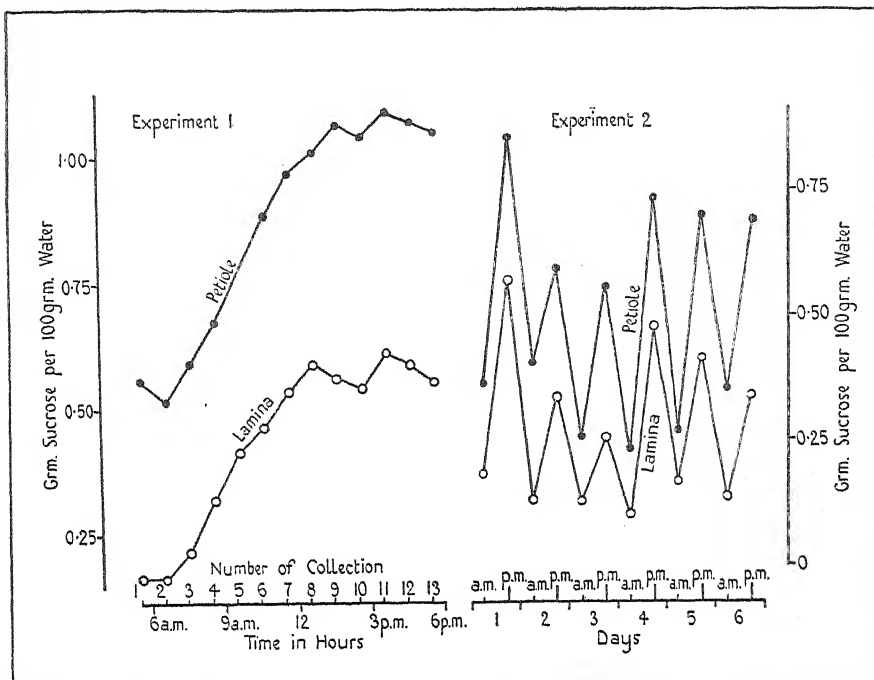
#### *Experiments 1 and 2.*

*Procedure.* The procedure for these experiments has already been described. Both are concerned with the diurnal changes in lamina and petiole, the first with hourly and the second with daily variations in the sugars. The reducing sugar concentrations are not reproduced, for as we have already shown, the fluctuations in the amount of reducing sugars in lamina and in petiole are either small and irregular, or else entirely absent.

*Results.* The results for sucrose are shown in Text-fig. 8. In Experiment 1 the total change in sucrose concentration during the day in the leaf was 0.46 (gram. per 100 gram. water), while in the petiole it was somewhat greater, amounting to 0.58. In Experiment 2, in which the collections were only made at intervals of approximately twelve hours, there is the difficulty that there must be some lag between the concentration changes in the lamina and in the petiole, but the results for Experiment 1 suggest that this lag must be small. It will be seen, however, that here too the diurnal increases in concentration in the petiole exceed those in the lamina. Thus for the petiole the mean increase was 0.388, while for the lamina it was only 0.277.

The presence of some mechanism, either in the petiole or in the vein, or in both, that can accumulate sucrose against a concentration gradient is thus demonstrated. The energy necessary must, of course, be released in metabolism. It seems evident that neither the *diffusion* nor the *druckstrom* hypothesis has contemplated the existence of such a mechanism. When it is recollected that nearly every cell in the lamina is engaged in the manufacture of sugar, while only a very small proportion of those of the petiole serve conduction, it seems probable that the diurnal increase in

the actual conducting tracts must be enormously greater than in the assimilating cells.



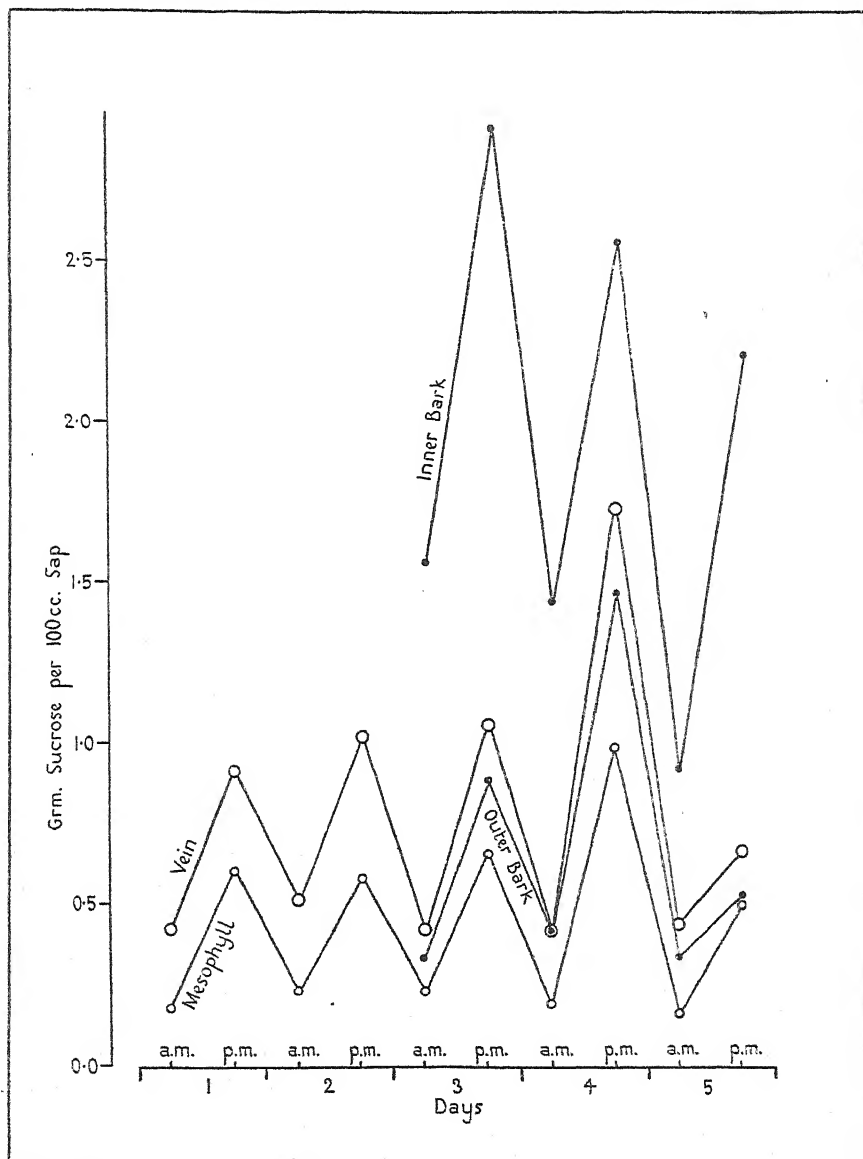
TEXT-FIG. 8. Hourly (Experiment 1) and daily (Experiment 2) variations in sucrose concentrations (grm. per 100 grms. water) of lamina and petiole.

#### *Experiment 4. December 17th, 1931.*

**Procedure.** In this experiment the primary veins were removed from the lamina. The latter (cf. p. 587) is for convenience referred to as mesophyll. Collections were made at 6.30 a.m. and 3 p.m. over a period of five days. For the last three days the petioles also were sampled. Petiolar bark and wood were separated, and the bark was subdivided into inner and outer regions. The whole of the phloem was contained in the inner region, which consisted approximately of 28 per cent. phloem, 30 per cent. fibres, and 42 per cent. ray parenchyma. The labour and time involved in separating the tissues of the petiole was so great that only one sample per collection could be taken. The results are expressed per 100 c.c. sap. The primary veins accounted for approximately 8 per cent. of the fresh weight of the lamina.

**Results.** The diurnal changes in sucrose concentration are shown in Text-fig. 9. The reducing sugar concentrations are again not reproduced, for the diurnal changes were small and irregular. The mean diurnal increases in concentration during the day for the last three days of the

experiment were as follows: mesophyll 0.56, vein 0.73, outer bark 0.60, inner bark 1.25 gm. per 100 c.c. sap. The diurnal increase in the inner



TEXT-FIG. 9. Daily variations in sucrose concentrations (gm. per 100 c.c. sap) of mesophyll, vein, inner and outer petiolar bark (Experiment 4).

bark is much greater than in the other tissues sampled, and as this is the region that contained the phloem, the inference is that the *phloem is the*

region where the accumulation of sucrose takes place. The cause of the large diurnal change in sucrose concentration in the outer bark of the petiole is not clear. If the phloem is the only tissue accumulating sucrose from the mesophyll, and it seems unlikely that more than one tissue should be responsible, then the increase in concentration during the day in the outer bark would suggest rapid leakage from the phloem while its concentration is high. The rapid fall during the night is as great or greater than that in the mesophyll, and presumably could not therefore all be due to respiration. Of the other possibilities that present themselves, and which will be considered later, accumulation and removal by the phloem from the cortex during the night, while its concentration is relatively low, seems the most probable.

*Experiment 5. December 12th, 1931.*

*Procedure.* In this experiment the effect of ringing on the sugar concentrations of the constituent tissues of the leaf is examined. The process of subdivision and the tissues sampled were similar to that of the previous experiment. One group of plants was ringed at ground level. There was only one sample per collection. The sequence of events is shown below.

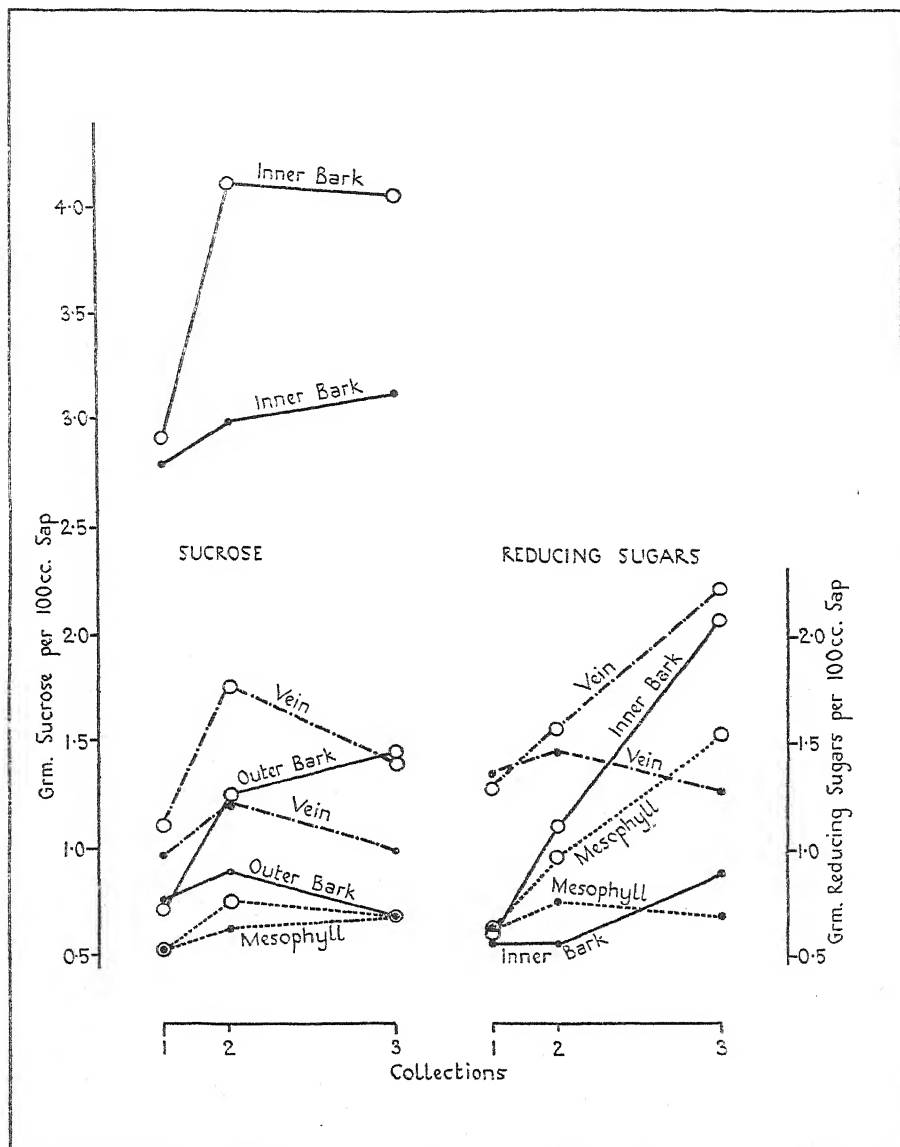
*Time-table.*

December	7.	8-9.0	a.m.	Ringing.
"		12.0	p.m.	1st ringed collection.
"		1.30	p.m.	1st normal collection.
"	9.	1.0	p.m.	2nd normal collection.
"		2.30	p.m.	2nd ringed collection.
"	14.	1.0	p.m.	3rd ringed collection.
"		2.0	p.m.	3rd normal collection.

*Results.* The concentrations of sucrose in the different tissues are shown on the left of Text-fig. 10. The reducing sugar concentrations, except that of the outer (petiolar) bark, are on the right. The initial sucrose response to ringing in the inner bark is much greater than that in any of the other tissues examined. The suggestion that the phloem is the region where concentration takes place is thus reinforced. It will be noted that the sucrose response is initially greatly in excess of the reducing sugar response in the inner bark. The great increase in reducing sugars occurs later. It would appear that sucrose is the sugar accumulated by the phloem, and that, after it reaches a certain concentration, it undergoes inversion. It is significant that the response of the mesophyll to ringing, both for sucrose and for reducing sugars, is smaller than that of any of the other tissues.

To sum up, the results recorded in this section show that the concentrations of sucrose in vein and petiole exceed that in the mesophyll. If sucrose is the form in which carbohydrates cross the mesophyll to the vein,

it would appear that it must be accumulated against a gradient. This, however, is not a necessary consequence of the negative gradient, as



TEXT-FIG. 10. Sucrose and reducing sugar concentrations (gm. per 100 c.c. sap) of mesophyll, vein, and inner petiolar bark and also sucrose concentrations of outer petiolar bark of normal and ringed leaves: normal •, ringed O. (Experiment 5).

a positive dynamic gradient might be masked by a steeper negative gradient of static sucrose. That this is not so is shown by the fact that

the increases in sucrose concentration in vein and in petiole during the day and also on ringing are greater than those in the mesophyll. Accumulation of sucrose against a gradient is thus indicated.

On subdividing the bark of the petiole into inner and outer regions, the former containing the whole phloem and the latter consisting mainly of cortical parenchyma, it was found that the concentration, and the *changes in concentration during the day and on ringing, in the inner region were much greater than those in the outer region, in the vein or in the mesophyll.* As this inner region contains the phloem, and as the phloem is continuous throughout petiole and vein, we infer that sucrose is accumulated by the phloem of the fine veins, and that it leaks into the vein parenchyma and petiolar cortex as it travels along the phloem towards the stem. The difficulty in this hypothesis is that the phloem in the fine vein is assumed to be concentrating sucrose, while elsewhere it is leaking away from it. At this stage it is necessary to consider the distribution of sieve-tubes and companion cells in the leaf.

#### SECTION 5. SIEVE-TUBE/COMPANION CELL RELATIONS.

Though the relations of sieve-tube and companion cell in the leaf of the cotton plant<sup>1</sup> are essentially those described by Fischer (17), yet the anatomy of the bundle-ends and anastomoses appears to differ in many important respects from his account. In the large veins the relative dimensions of sieve-tube and companion cell are approximately similar to those in petiole and stem; the companion cells are much smaller than the sieve-tubes, and the total area occupied by them amounts to only a small proportion of that occupied by the sieve-tubes. The individual elements are much smaller than in the stem.

As the bundle-ends are approached the sieve-tubes gradually diminish in cross-sectional area and the companion cells are greatly enlarged, so that the sieve-tubes come to represent only a small proportion of the area occupied by the companion cells. In the leaf of the cotton plant sieve-tubes are present as long as there is phloem, and undivided mother-cells, similar in all respects to the large companion cells, are present for some distance away from the bundle-ends. The phloem of the fine veins therefore consists of small sieve-tubes with large companion cells, and also large undivided mother-cells that resemble companion cells. Fischer refers to both as *Transition* cells. Phloem parenchyma is not present. A typical cross section of a fine vein, with two small sieve-tubes embedded in seven large cells with somewhat dense contents, is shown in Plate XXIII, fig. 2.

<sup>1</sup> Work is at present in progress on the phloem anatomy of the foliage leaf by M. C. Jardine, to whom we are indebted for information contained in this section.



The fine veins and bundle-ends are sheathed by a single layer of large elongated parenchyma, the *Border parenchyma*. In the large veins this is replaced by the massive *Nerve parenchyma* (Plate XXIII, fig. 3).

The proportion of phloem and jacketing parenchyma also varies greatly. In the very fine veins with thin border parenchyma the cross-sectional area of the phloem may amount to 20 per cent. of the whole vein, while at the base of the primary vein it sinks to about 4 per cent. The fine veins are characterized therefore, by a single layer of border parenchyma and a large proportion of phloem, consisting of the companionlike transition cells and small sieve-tubes. The large veins on the other hand, contain a massive sheath of nerve parenchyma and a small proportion of phloem, containing phloem parenchyma and fibres in addition to large sieve-tubes and small companion cells.

If, as we have suggested, the phloem of the fine veins is responsible for the accumulation of sucrose against a gradient, it seems reasonable to assume that the transition cells are the agents concerned. Sucrose might move across the mesophyll to the border parenchyma down a gradient, while the transition cells remove it from the border parenchyma against a gradient. After accumulation by the transition cells, sucrose would be liberated into the sieve-tubes and thence be distributed throughout the plant. Polar distribution of sucrose by the transition cells is suggested.

If we assign to the transition cells the function of accumulating sucrose against a gradient, we must assume that the companion cells, from which they are indistinguishable, throughout the length of the phloem can also accumulate sucrose from the parenchyma with which they are in contact. As we have seen (cf. Text-fig. 9) the concentration of sucrose in the cortical parenchyma (outer bark) of the petiole rises by day and falls by night. If the rise by day is due to leakage from the sieve-tubes, then leakage must occur at the same time that the companion cells are engaged in accumulation. Leakage from the sieve-tubes and accumulation by the companion cells may in fact take place simultaneously throughout the phloem. In the fine veins the large proportion of companion cells would ensure that accumulation predominates and that the net movement is from mesophyll to phloem.

In the petiole on the other hand with its relatively small proportion of companion cells, increased leakage from the sieve-tube<sup>1</sup> during the day, consequent on the steepening of the gradient from sieve-tube to cortex, may be more than the accumulating cells can deal with. There would thus be leakage of sugar into the cortical parenchyma. During the night, leakage from the sieve-tubes would fall off considerably because of the diminished concentration. In this case the companion cells might not only

<sup>1</sup> The absence of phloem parenchyma from the fine vein and its presence in the petiole may be an additional factor.

be able to deal with all leakage, but might also be able to regain some of the material which leaked into the cortex during the day.

Thus a difference between the functional activity of the phloem of the fine veins and that of the petiole may result from the fact that the transition cells in the fine veins are not normally working at full capacity, while the companion cells of the petiole are.

#### SECTION 6. VEIN GRADIENTS (EXPERIMENT 6).

Our suggestion that the transition cells are responsible for the accumulation of sucrose from the mesophyll, or more accurately from the border parenchyma, assumes that there is a sudden step-up in sucrose concentration in passing from the mesophyll, or rather the border parenchyma, to the phloem of the fine veins, where the transition cells are mainly located, and that the head for sugar distribution throughout the plant is consequently located in these cells. The work of Mangham (28), however, indicates that the accumulation of sugar in the phloem of the vein may be a gradual process. He studied by means of osazones the distribution of sugar in leaves which had been darkened for some days, and found that the concentration was greater in the sieve-tubes of the large than in those of the fine veins. The presence of a negative gradient in the sieve-tubes of the vein, if established, would indicate that there is not a sudden change in concentration in the region of the fine veins, and that the process of accumulation is consequently a gradual one. Decision as to differences in concentration which are based only on microchemical methods must, however, be accepted with reserve.

Unfortunately, we cannot subdivide the tissues of the vein in the same way that the petiole and the stem can be subdivided. Calculation of the concentrations in the phloem of the vein is thus impossible. Moreover, we are unable to separate the very fine veins, where accumulation is assumed to take place, from the lamina. Under the circumstances we have resorted to extrapolation from the concentrations found in larger veins which can be separated from the lamina. It will be evident that if the concentration of sucrose in the fine vein as a whole exceeds that in the adjacent mesophyll, accumulation by the fine veins would be indicated, and the assumption of a gradual accumulation of sucrose along the phloem of the vein would not be necessary.

#### *Experiment 6. January 20th, 1932.*

As much as possible of the primary veins were removed from the lamina. They were then divided into three portions of approximately equal length. The very fine apical part with border parenchyma could not of course be separated from the lamina, but it was estimated that the

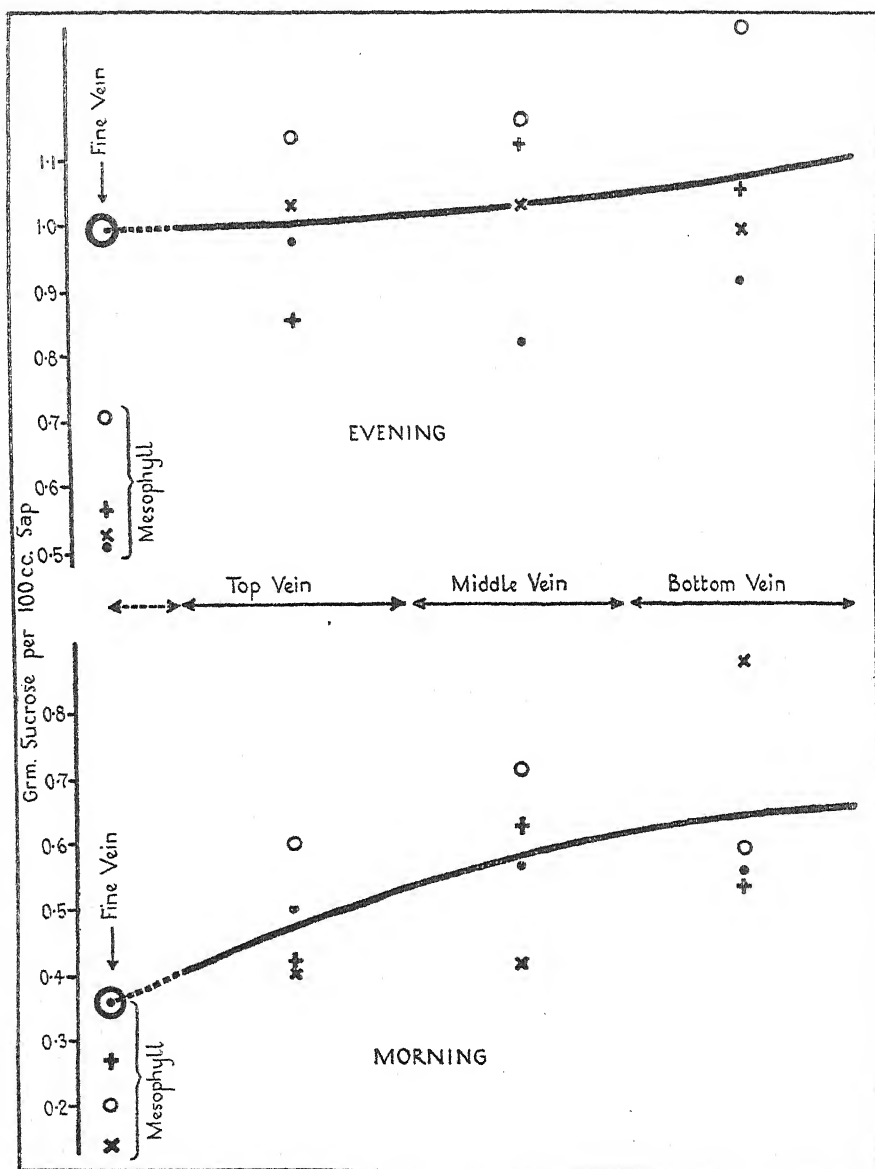
distance from this region to the end of the smallest veins sampled was not more than one-tenth of that of the whole vein taken. The experiment lasted for four days and collections were made at 6.30 a.m. and 3 p.m. on each day. Only one sample per collection could be taken as the labour and time involved in removing the veins was so great.

*Results.* The morning and the evening results for the four days are recorded graphically in Text-figs. 11a and 11b. The evening values are of course greater than those of the morning. The values for the individual days are also shown. The calculated concentrations in the fine veins are indicated by the circle above the values for the mesophyll. They are obtained by extrapolation from curves fitted to the mean points for each vein section, assuming these to be of the type  $y = ax^2 + bx + c$ . It is of course uncertain to what extent extrapolation is justified, for there is a rather rapid change in the ratio of area of companion cell to sieve-tube as the fine vein is approached. The deviation from the mean values, especially for the vein, are considerable, and discretion must be used in comparing the extrapolated values for the fine veins with those of the mesophyll. The extrapolated values for the fine veins, however, are in every case greater than those of the mesophyll. It would thus appear that the concentration both of sucrose and of reducing sugars in the fine veins is much higher than in the mesophyll, especially during the day; and, consequently, that there is a sudden change in sugar concentration in this region. It will be noticed that there is a suggestion of a negative gradient along the vein. If the phloem gradients in the vein are positive, it would then follow that the gradients in the sheath and wood must be markedly negative. The concentration in the border parenchyma might well be less than that of the mesophyll, so that movement from mesophyll to border parenchyma would be *with* the gradient and transport from the border parenchyma to phloem *against* the gradient.

Summing up, our estimates of the sugar concentration of the fine veins indicate that the accumulation of sucrose is not a gradual process, as Mangham's observations suggest, but that it is sudden and is probably localized in the phloem of the fine veins.

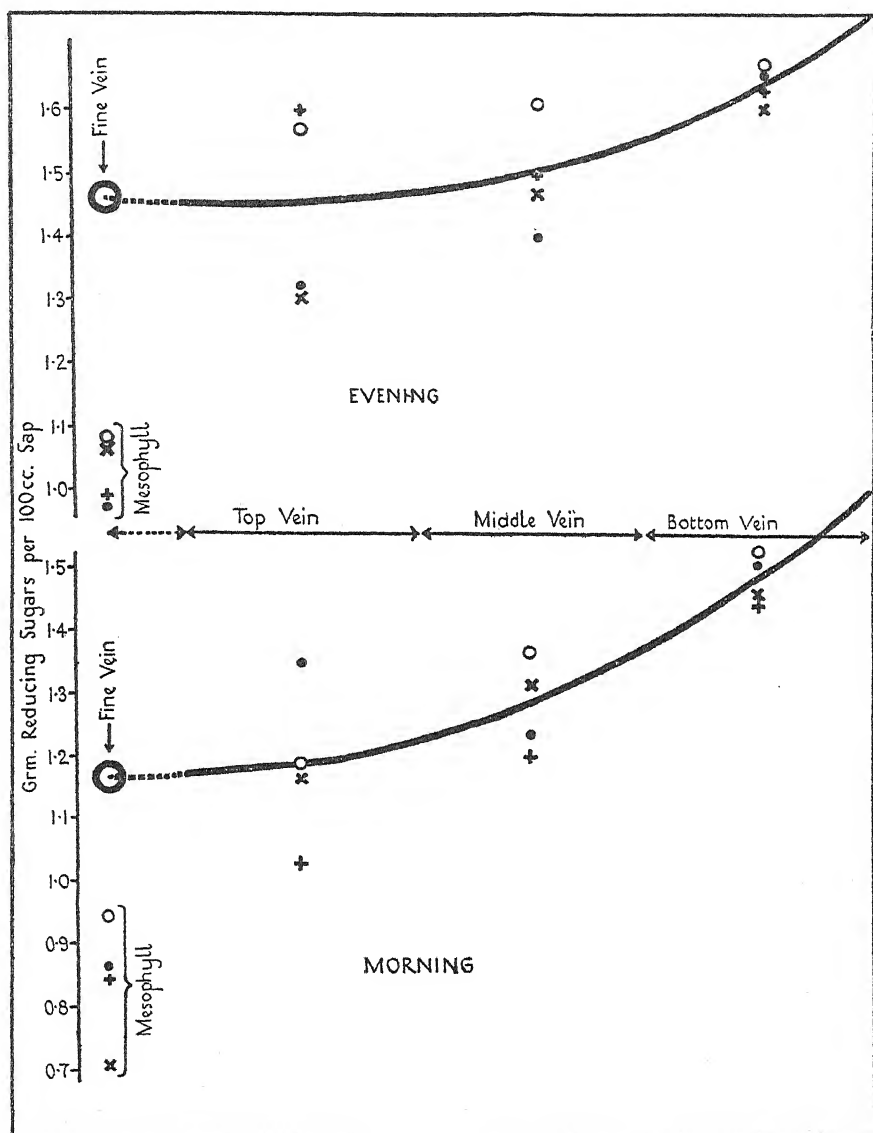
#### SECTION 7. PHLOEM GRADIENTS (EXPERIMENT 5).

While a *Diffusion* theory of transport requires a positive sugar gradient in the phloem of the vein during export, and though the evidence advanced in the last section in favour of a sudden rather than a gradual accumulation of sugar by the vein suggests a positive rather than a negative gradient in the vein phloem, yet the existence of such a positive gradient has not been demonstrated. As already remarked, separation of the tissues of the vein has not yet proved practical, so that no estimate can be made of the concentration in the phloem of the vein by means of the methods



TEXT-FIG. 11A. Morning and evening concentrations (grm. per 100 c.c. sap) of sucrose on four successive days in three regions along the primary vein and in the mesophyll, with lines of closest fit and extrapolated concentrations for fine veins (Experiment 6).

● 1st day; + 2nd day; ○ 3rd day; x 4th day.



TEXT-FIG. 11 B. Morning and evening concentrations (gm. per 100 c.c. sap) of reducing sugars on four successive days in three regions along the primary vein and in the mesophyll, with lines of closest fit and extrapolated concentrations for fine veins (Experiment 6).

● 1st day; + 2nd day; ○ 3rd day; × 4th day.

adopted by Mason and Maskell for the phloem of the stem. Their estimates of phloem concentration indicate that along the stem the direction of transport is from a region of high to one of low concentration, but it does not of necessity follow, though it is of course very probable, that movement in the phloem from one organ to another is also along a concentration gradient. If, however, it could be demonstrated that transport from the petiole to the stem, for instance, was along a positive gradient in the phloem, it might encourage belief in the existence of a positive gradient also in the phloem of the vein. In the present section we have attempted therefore to estimate, and to compare, the concentrations in the phloem of the petiole and of the stem. Calculations of the concentrations in the phloem are also of importance in order that the concentrations in phloem and mesophyll may be compared.

*Experiment 5. December 12th, 1931.*

*Procedure.* Reference has already been made (cf. p. 614) to this experiment in connexion with the effect of ringing on the concentrations of sugar in the various parts of the leaf. The tissues sampled consisted not only of mesophyll, vein, outer and inner petiolar bark, but also of the bark from the stem between the foliage region and the root. The bark from the stem was subdivided into outer, middle, and inner regions. Reference has been made in the section on methods to the procedure adopted in calculating the phloem concentrations.

*Results.* The concentrations in the mesophyll and those calculated for the phloem of petiole and of bark are shown in Table X. The results for the three normal and three ringed collections are shown separately in the table. The mean concentrations in the ringed group are generally in excess of those in the normal group. The presence of the polyglucoside in the phloem is very doubtful, and it has therefore been omitted from the table. To take first of all the sucrose concentrations, it will be seen that the mean concentrations in the petiolar phloem are about ten times those in the mesophyll and are also greater than those in the phloem of the stem. If our estimated concentrations have any basis in fact, there is therefore a positive gradient from the petiolar to the stem phloem. Furthermore, if we may infer from this that the gradient from the vein to the petiolar phloem is also positive, the negative gradient from mesophyll to the phloem of the fine veins must be enormous.

The results for the hexoses are chiefly of interest in that they show no consistent gradients from the mesophyll to the phloem of petiole and of stem. The mean hexose concentrations in the mesophyll of both normal and ringed groups are above those in the stem phloem, but are considerably below those in the petiolar phloem. It must be remembered that the hexose concentrations in the mesophyll are probably overestimates

TABLE X.

*Concentrations (grm. per 100 c.c. sap) in Mesophyll and Estimated Concentrations in Phloem of Petiole and Stem.*

		Mesophyll.			Petiolar phloem.			Stem phloem.			
		Sucrose.	Glucose.	Fructose.	Polyglucoside.	Sucrose.	Glucose.	Fructose.	Sucrose.	Glucose.	Fructose.
Normal	1.	0.52	0.37	0.24	0.37	5.87	0.28	0.33	4.40	0.50	-0.36
	2.	0.62	0.54	0.19	0.17	6.14	0.74	0.00	5.81	-0.15	0.31
	3.	0.68	0.40	0.26	0.32	6.78	0.58	0.60	5.68	0.30	-0.17
	Mean	0.61	0.44	0.23	0.29	6.26	0.53	0.31	5.30	0.22	-0.07
Ringed	1.	0.51	0.25	0.35	0.29	6.26	0.82	0.00	4.77	0.34	-0.34
	2.	0.74	0.51	0.44	0.23	8.37	1.97	0.00	7.02	0.00	0.49
	3.	0.68	0.99	0.53	0.21	7.93	0.57	1.45	7.07	0.44	0.08
	Mean	0.64	0.58	0.44	0.24	7.52	1.12	0.48	6.29	0.26	0.08

due to the reducing power of the polyglucoside, which would make the actual gradients more negative. There is, on the average, a positive gradient in both glucose and fructose from the petiolar to the stem phloem.

To sum up, it appears probable that the sucrose, glucose, and fructose gradients from mesophyll to the phloem of the fine veins are all markedly negative. Sucrose, which previous work has pointed to as the form in which carbohydrates travel from mesophyll to vein, must consequently be accumulated against an enormous sucrose gradient.

#### SECTION 8. REVERSAL OF NORMAL DIRECTION OF TRANSPORT (EXPERIMENT 7).

Mason and Maskell (33) remark 'if sugar transport in the plant is analogous, apart from questions of absolute rate, to movement by physical diffusion, then it ought to be possible by judicious manipulation to reverse the normal direction of transport'. In the stem they succeeded in reversing the normal downward movement of carbohydrate transport, and showed that the reversal of the direction of movement was accompanied by a reversal of the sugar gradient in the inner part of the bark, where the phloem, of course, is mainly located. They also attempted to make leaves import carbohydrate. Some of the leaves on a number of plants were darkened and the stems were ringed. The rest of the leaves were illuminated, so that carbohydrate ought, if gradients determine the direction of transport, to have travelled from the illuminated to the darkened leaves. They found that the import, if any, was very small, even though the increase in sugar concentration in the bark of the stem was considerable.

It will be evident that if the phloem of the vein can accumulate sugar from the mesophyll against a gradient, there is an expenditure of energy altogether apart from that used in accelerating diffusion along the sieve-tube. It is at present uncertain to what extent the veins can drain the mesophyll of its sugar, but if the machinery of accumulation is infinitely efficient at all concentrations and can deprive the mesophyll of the whole of its sugar, then all backward movement from vein to mesophyll would be impossible. Furthermore, if the accumulation of sugar occurs only in the fine veins, and not throughout the length of the vein, it ought to be possible to make the large veins import sugar even though import by the mesophyll is impossible. If this was demonstrated, there would be additional grounds for assuming that the fine veins, and therefore the transition cells, are responsible for the accumulation of sugar from the mesophyll, or rather from the border parenchyma, for sucrose should travel from mesophyll to vein by a process analogous to diffusion.



*Experiment 7. December 16th, 1931.*

*Procedure.* In order to produce conditions likely to bring about backward movement into the lamina, only 10 per cent. of the leaves on each plant were darkened. As a preliminary these leaves were covered with paper bags in order to diminish the concentration of sugar in the mesophyll. The paper bags were white externally and blackened on the inside. The rest of the leaves were, of course, normally illuminated. As soon as it was judged that the sugar concentration in the darkened leaves had dropped sufficiently, the stems were ringed. The ringing was done in order to raise the concentration of sugar in the illuminated leaves, and so render the export of sugar from the illuminated to the darkened leaves more probable.

Both illuminated and darkened leaves were sampled. They were subdivided into mesophyll, primary veins, petiolar wood, and petiolar bark. The latter was divided into inner and outer regions. Only one sample of fifty leaves per collection could be undertaken. Fresh weight and moisture determinations were made only on the mesophyll and veins, while sugar concentrations were determined on all tissues sampled. The sequence of events was as follows:

*Time-table.*

Feb. 15,	1931.	2.0 p.m.	Bags placed on leaves.
" 16,	"	8.30 a.m.	Ring of plants.
" 16-19,			Collections of illuminated and darkened leaves at 7 a.m. and 3.30 p.m.

*Results.* As there was only one sample per collection, the significance of the average change with time in dry weight of vein and mesophyll is not easy to assess. We have accordingly followed the procedure of Mason and Maskell (33), and calculated the correlation coefficients between the dry weight per sample and the time interval that elapsed from the time of ringing. These are shown in Table XI.

TABLE XI.

*Correlation Coefficients between Dry Weights per Sample of Mesophyll and Vein and the Time Interval Elapsing from the Time of Ringing.*

Illuminated	{ Mesophyll	0.911
	{ Vein	0.940
Darkened	{ Mesophyll	0.516
	{ Vein	0.804

The correlations are all positive and all are significant, with the exception of that of the darkened mesophyll. An import of carbohydrate by the darkened vein is thus indicated, while entry into the darkened

due to the reducing power of the polyglucoside, which would make the actual gradients more negative. There is, on the average, a positive gradient in both glucose and fructose from the petiolar to the stem phloem.

To sum up, it appears probable that the sucrose, glucose, and fructose gradients from mesophyll to the phloem of the fine veins are all markedly negative. Sucrose, which previous work has pointed to as the form in which carbohydrates travel from mesophyll to vein, must consequently be accumulated against an enormous sucrose gradient.

#### SECTION 8. REVERSAL OF NORMAL DIRECTION OF TRANSPORT (EXPERIMENT 7).

Mason and Maskell (33) remark 'if sugar transport in the plant is analogous, apart from questions of absolute rate, to movement by physical diffusion, then it ought to be possible by judicious manipulation to reverse the normal direction of transport'. In the stem they succeeded in reversing the normal downward movement of carbohydrate transport, and showed that the reversal of the direction of movement was accompanied by a reversal of the sugar gradient in the inner part of the bark, where the phloem, of course, is mainly located. They also attempted to make leaves import carbohydrate. Some of the leaves on a number of plants were darkened and the stems were ringed. The rest of the leaves were illuminated, so that carbohydrate ought, if gradients determine the direction of transport, to have travelled from the illuminated to the darkened leaves. They found that the import, if any, was very small, even though the increase in sugar concentration in the bark of the stem was considerable.

It will be evident that if the phloem of the vein can accumulate sugar from the mesophyll against a gradient, there is an expenditure of energy altogether apart from that used in accelerating diffusion along the sieve-tube. It is at present uncertain to what extent the veins can drain the mesophyll of its sugar, but if the machinery of accumulation is infinitely efficient at all concentrations and can deprive the mesophyll of the whole of its sugar, then all backward movement from vein to mesophyll would be impossible. Furthermore, if the accumulation of sugar occurs only in the fine veins, and not throughout the length of the vein, it ought to be possible to make the large veins import sugar even though import by the mesophyll is impossible. If this was demonstrated, there would be additional grounds for assuming that the fine veins, and therefore the transition cells, are responsible for the accumulation of sugar from the mesophyll, or rather from the border parenchyma, for sucrose should travel from mesophyll to vein by a process analogous to diffusion.

*Experiment 7. December 16th, 1931.*

*Procedure.* In order to produce conditions likely to bring about backward movement into the lamina, only 10 per cent. of the leaves on each plant were darkened. As a preliminary these leaves were covered with paper bags in order to diminish the concentration of sugar in the mesophyll. The paper bags were white externally and blackened on the inside. The rest of the leaves were, of course, normally illuminated. As soon as it was judged that the sugar concentration in the darkened leaves had dropped sufficiently, the stems were ringed. The ringing was done in order to raise the concentration of sugar in the illuminated leaves, and so render the export of sugar from the illuminated to the darkened leaves more probable.

Both illuminated and darkened leaves were sampled. They were subdivided into mesophyll, primary veins, petiolar wood, and petiolar bark. The latter was divided into inner and outer regions. Only one sample of fifty leaves per collection could be undertaken. Fresh weight and moisture determinations were made only on the mesophyll and veins, while sugar concentrations were determined on all tissues sampled. The sequence of events was as follows:

*Time-table.*

Feb. 15,	1931.	2.0 p.m.	Bags placed on leaves.
" 16,	"	8.30 a.m.	Ring of plants.
" 16-19,			Collections of illuminated and darkened leaves at 7 a.m. and 3.30 p.m.

*Results.* As there was only one sample per collection, the significance of the average change with time in dry weight of vein and mesophyll is not easy to assess. We have accordingly followed the procedure of Mason and Maskell (33), and calculated the correlation coefficients between the dry weight per sample and the time interval that elapsed from the time of ringing. These are shown in Table XI.

TABLE XI.

*Correlation Coefficients between Dry Weights per Sample of Mesophyll and Vein and the Time Interval Elapsing from the Time of Ringing.*

Illuminated	Mesophyll	0.911
	Vein	0.940
Darkened	Mesophyll	0.516
	Vein	0.804

The correlations are all positive and all are significant, with the exception of that of the darkened mesophyll. An import of carbohydrate by the darkened vein is thus indicated, while entry into the darkened

mesophyll is doubtful. The regression lines of dry weight on time are shown in Text-fig. 12. It will be seen that in the illuminated leaf the average change with time is greater for the mesophyll than for the vein, while the reverse obtains for the darkened leaf. The initial differences in the dry weights of the two groups are presumably due to the preliminary starvation of the darkened leaves and not to sampling, for extreme precautions were taken in the grading of the leaves. A difference in the ease of import by the mesophyll and the vein of the darkened leaves is suggested. Some import by the darkened mesophyll is indicated, as in spite of respiration there was no loss in dry weight. Consideration of the relative changes in dry weight emphasizes this difference in ease of entry into vein and mesophyll. The relative changes are shown in Table XII.

Import of sugar is of course only indicated into the *primary* veins, in which interchange of sugar between phloem and the surrounding tissues occurs readily, for the proportion of sieve-tube and companion cell here is approximately the same as that of petiole and stem. Import into the *sieve-tubes* of the fine veins might also occur, but it seems unlikely that the transition cells, which compose the greater part of the phloem in this region of the vein, would allow of leakage into the border parenchyma. The small import of carbohydrate that apparently did take place into the mesophyll of the darkened leaf may have occurred in the region of the larger veins.

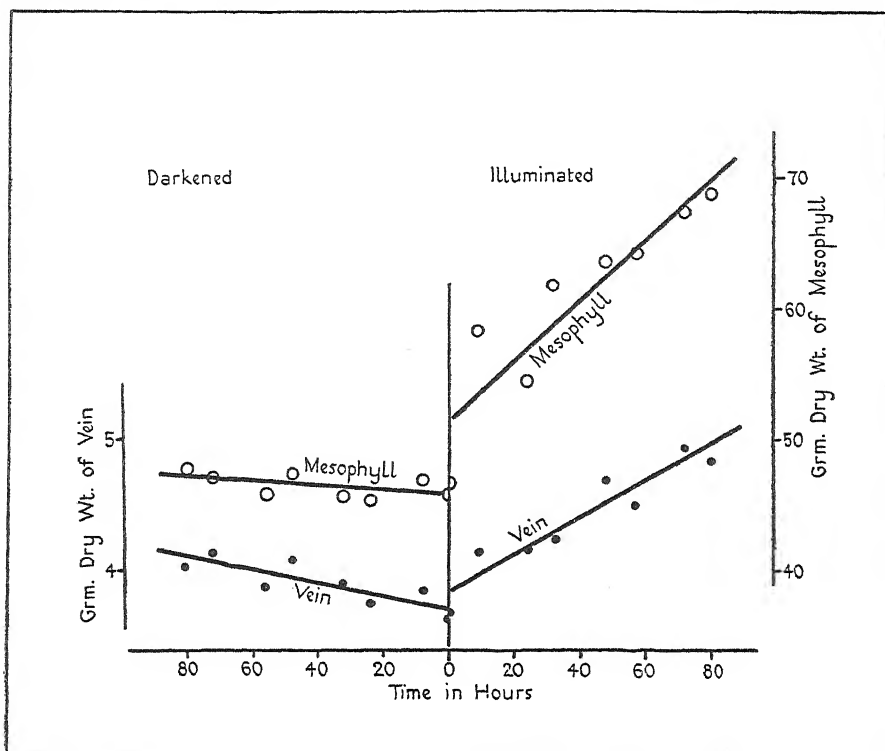
TABLE XII.

*Percentage Increase of Final Dry Weight of Mesophyll and Vein on Initial Dry Weight.*

Illuminated	Mesophyll	47.15
	Vein	30.89
Darkened	Mesophyll	4.53
	Vein	10.44

The concentrations of *total* sugars after invertase inversion are shown in Text-fig. 13. The curves suggest the following sequence of events. Ringing the stem resulted in a rapid increase in the sugar concentration of the phloem throughout the plant. This is reflected in the great increase in concentration in the inner petiolar bark. The increase in concentration in the phloem of the veins of the illuminated leaves led to a diminished rate of export from the mesophyll, and a consequent increase in its concentration. A subnormal rate of export from the mesophyll would result from the steepening of the negative gradient between mesophyll and the transition cells, and more energy would be expended in the concentration of unit weight of sugar. This would, however, be rapidly adjusted as the concentration rose in the mesophyll, and the normal or nearly normal gradients and rates of export might obtain.

After the second collection the concentration in the illuminated mesophyll ceased to rise, for the rate of conversion of sugar into reserve carbohydrate, respiration and export overtook sugar production. It will be

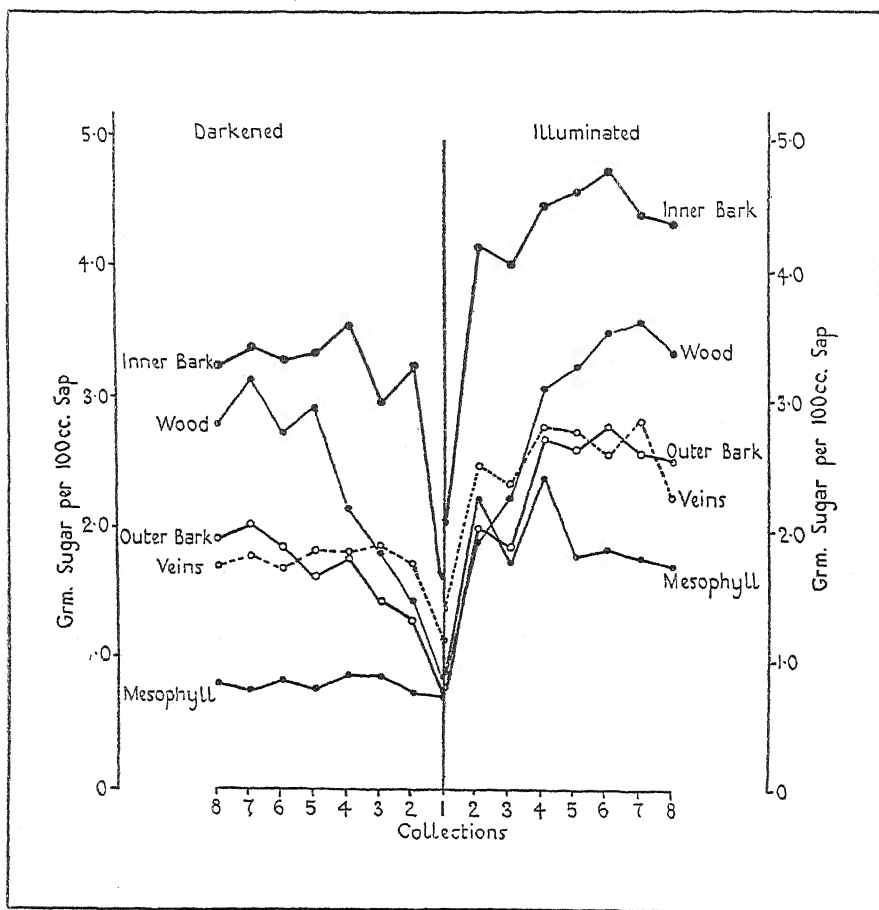


TEXT-FIG. 12. Regression lines of dry weight (per 50 leaves) on time for mesophyll and veins of illuminated and darkened leaves (Experiment 7).

noticed that diurnal changes in the concentration of the illuminated mesophyll were only manifested for two days. The arrest of further increase in concentration in the mesophyll is reflected in the inner bark of the petiole, which contains the whole of the phloem. The other tissues of the illuminated leaf then achieved dynamic equilibrium with the phloem, some rapidly and others more slowly. Thus the whole vein came very rapidly into equilibrium with its phloem, while the attainment of equilibrium in the petiolar wood was slower.

In the darkened leaf the changes in the inner petiolar bark, and therefore the phloem, show a striking agreement with those of the inner bark of the illuminated leaf. The other tissues, with the exception of the mesophyll, which remained unchanged, come into equilibrium after varying periods. As in the illuminated leaf, the vein rapidly achieved equilibrium, the wood was slower, and the outer petiolar bark was intermediate.

The difference in the behaviour of the mesophyll and the vein is best illustrated by considering the ratios of the concentrations in the phloem, as represented by the inner bark, to the concentrations in other tissues.



TEXT-FIG. 13. Concentrations (gram. per 100 c.c. sap) of total sugars in mesophyll, veins, inner and outer petiolar bark and wood of illuminated and darkened leaves (Experiment 7).

Inspection of the graph indicates that all the tissues had reached approximate equilibrium by the sixth collection. We have therefore taken the mean concentration for the last three collections as representing the concentration when dynamic equilibrium with the phloem was reached, and have expressed (Table XIII) the mean concentration in each tissue as a percentage of the mean concentration in the inner bark.

Inspection of the table reveals a close agreement between the values for the veins and for the outer bark in the two groups. The ratio in the wood of the darkened petiole is somewhat greater than would be expected

from that of the illuminated group. Of major importance is the fact that the concentration in the mesophyll of the darkened leaf is much less than would be expected, if only change in gradient determined movement between mesophyll and phloem. This is tolerably clear, even from inspection of the graph. It reinforces the conclusions drawn from the dry weights, and demonstrates that, although concentration gradients may determine the direction of movement along the larger veins, yet there are other factors present that determine the direction of transport between mesophyll and the fine veins.

TABLE XIII.

*Mean Concentrations for Collections 6, 7, and 8 in Mesophyll, Veins, Petiolar Wood, and Outer Bark as Percentages of Mean Concentration in Inner Bark.*

		Illuminated.	Darkened.
Lamina	Mesophyll	39.6	24.4
	Vein	56.9	52.4
Petiole	Outer bark	58.6	58.7
	Wood	77.7	87.4

## SECTION 9. DISCUSSION.

That physical diffusion is much too slow to distribute carbohydrates throughout the plant body has long been recognized. Mason and Maskell (33) calculated that transport in the phloem was from 20,000 to 40,000 times greater than the rate of physical diffusion. To avoid this difficulty Dixon (16) suggested a reversal of the transpiration current in the peripheral regions of the wood, and invoked tension differences to ensure the circulation of sap. Münch (37) also suggested a mass movement of solution, but in the sieve-tubes, and invoked differences in hydrostatic pressure as the motive force. Much earlier de Vries (15) had suggested that diffusion might be accelerated in the sieve-tubes by means of protoplasmic streaming. Very recently (34) it has been pointed out that the rate of this streaming would have to be greatly in excess of the highest observed rates of streaming, and that ebb and flow currents of enormous velocity would have to obtain in the sieve-pores. That materials travel in the sieve-tubes by some such process involving independent movement of solutes, and not by a mass movement of solution, is, we think, very probable. However this may be, the outstanding fact brought to light in the present paper is that the transport of carbohydrates is confronted by a further difficulty, namely the accumulation of sugar by the phloem against a concentration gradient. The physical difficulties here are quite as great as those presented by the enormous rates of movement achieved

in the sieve-tubes, Energy relations, presumably dependent on carbohydrate metabolism (10), are doubtless involved by both phenomena.

The picture of carbohydrate export from the leaf suggested by the foregoing experiments is as follows: Of the four sugars believed to be present in the leaf, glucose, fructose, sucrose, and the polyglucoside, only one, sucrose, is concerned with transport across the mesophyll and is mainly responsible for movement throughout the sieve-tube system. Carbohydrate transport may thus fall into line with nitrogen transport (30), in that a single compound is responsible for movement from the assimilating cell to the other parts of the plant. The movement of sucrose across the mesophyll and into the border parenchyma of the bundle-ends and anastomoses is down a gradient. The movement from the border parenchyma into the phloem is against a gradient. Inasmuch as the concentration of sucrose appears to be much greater in the phloem than in any other tissue of the leaf, and as it seems improbable that more than one tissue can be concerned with the accumulation of sucrose against a gradient, the phloem is suggested as the region of accumulation. Moreover, as movement from mesophyll to phloem must occur in the fine veins, and as the phloem here is almost entirely composed of the specialized transition cells, we have suggested that these cells are particularly concerned with the accumulation of sucrose, and that sucrose is released thence into the sieve-tubes and distributed throughout the plant.

It will be evident that the accumulation of sucrose by the transition cell is polarized (cf. 42); sugar is not accumulated from the sieve-tube. In this respect an analogy may be sought in the accumulation of mineral elements by the root. The concentration is much greater in the living cells of the root than in the soil solution. It is also greater than in the dead cells of the wood, yet apparently accumulation from the sap in the tracheae does not occur, though certain elements (e.g. calcium) are doubtless accumulated against a gradient in the stem by the wood parenchyma from the transpiration stream. We have already extended the original idea of the transition cells being capable of accumulation to include the companion cells. Should this surmise have any basis, transport in the *phloem*, though not in the *sieve-tube* system, might be polarized, in that movement would occur from a region with a relatively large area of companion cells to a region poor in these elements. Moreover, the movement of carbohydrate stored in the stem into the phloem (9, 53) of trees in spring may depend on the presence of companion cells. In the yam (31) there are peculiar balls of parenchyma cells, the bast glomeruli, which interrupt the sieve-tubes at every node. The rates of carbohydrate transport in the yam are known to be exceptionally high (35), and it may be that the function of the bast glomeruli is to accumulate sugar from the sieve-tubes of one internode and to release it into the sieve-tubes of the



next; accumulation may in fact be polarized. We have found that the concentration of sugar at the node exceeds that of the adjacent internodes.

The mechanism of accumulation of mineral elements by plant cells is still a matter of debate. It does not even appear to be decided whether ions or undissociated molecules are mainly involved. That a non-electrolyte like sugar can be accumulated against a gradient by the fine veins perhaps suggests that electrical forces are not at work. In this connexion it is still uncertain whether root-cells can absorb sugar against a gradient and whether the fine veins of the leaf accumulate the inorganic elements that are exported through the phloem. Work on the gradients is at present in progress. It seems probable, however, that movement out of the leaf is polarized, even if there is not actual accumulation, for we have observed that when the supply of nitrogen to the root, and therefore the leaf, is curtailed, nitrogen continues to leave the leaf and to pile up in the stem tissues. It seems clear that sugars must enter young leaves via the phloem, and that the machinery of accumulation by the vein is developed later. It is possible that the abscission of the leaf may not be unconnected with the polar movement of solutes out of the lamina, for abscission is heralded by the 'ruckgang' of certain elements, notably nitrogen, phosphorus, and potassium.

#### SECTION 10. SUMMARY.

1. The sugars found in the foliage leaf of the cotton plant include the hexoses, glucose and fructose, sucrose, and an unknown polyglucoside. The polyglucoside is present only in very small amounts in the petiole and in the vein. In the stem tissues it appears to be absent. It is soluble in water and in 80 per cent. alcohol. It seems to possess considerable reducing power, which is approximately doubled on hydrolysis. It, or some similar substance, has been found in the leaves of a number of other plants.

2. It is concluded that sucrose is the chief form in which carbohydrates travel from the assimilating cell to the phloem of the bundle-ends and anastomoses, and also longitudinally through the phloem to the stem, &c. The grounds for this conclusion are: (a) it is the only sugar to show well-marked and consistent diurnal changes in the lamina and in the petiole, and these changes are accompanied by similar changes in the rate of transport; (b) the response in lamina and in petiole to interrupting transport by ringing the stem is much more rapid and more marked for sucrose than for the other sugars; and finally (c) on darkening isolated leaves there is a rapid loss of sucrose from the lamina and a gain by the petiole. Glucose also disappears from the lamina, but much more slowly

than sucrose. Fructose and the polyglucoside are less affected by darkening.

3. Estimates were made of the concentrations of sucrose in the phloem of the petiole and of the stem. These estimates indicate that the longitudinal sucrose gradient in the phloem is positive, and that consequently the concentration in the phloem of the fine veins is even higher than in the phloem of the petiole. As the sucrose concentration in the phloem of the petiole is about ten times as great as that in the mesophyll, it is inferred that the concentration in the phloem of the fine veins is enormously greater than that in the mesophyll. Estimates of the sucrose concentration in the fine veins lend support to this inference, and point to a sudden change in sucrose concentration between the mesophyll and the fine veins.

4. That sucrose is accumulated by the phloem of the fine veins from the mesophyll against a gradient of *mobile* sucrose is demonstrated by the observation that not only is the actual gradient negative, but the diurnal fluctuations in sucrose concentrations are much greater in the phloem than in the mesophyll. Moreover, when export from the leaf is checked by ringing the stem, the increase in concentration in the phloem is greatly in excess of that in the mesophyll.

5. Evidence that interchange of sugar between mesophyll and vein is polarized is supplied by an experiment in which a small proportion of the leaves on a plant were darkened. It was found that sugar entered much more readily into the veins than into the mesophyll of the darkened leaves.

6. As movement of sugar from mesophyll to phloem must occur mainly in the region of the fine veins with their single layered border parenchyma, and as the phloem here consists predominantly of transition cells, it is suggested that these elements are peculiarly associated with the polar accumulation of sucrose and its release into the sieve-tube. It is suggested that the function of the companion cells, which are essentially indistinguishable from transition cells, is also to accumulate sucrose from the adjacent parenchyma and release it into the sieve-tubes.

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## EXPLANATION OF PLATE XXIII.

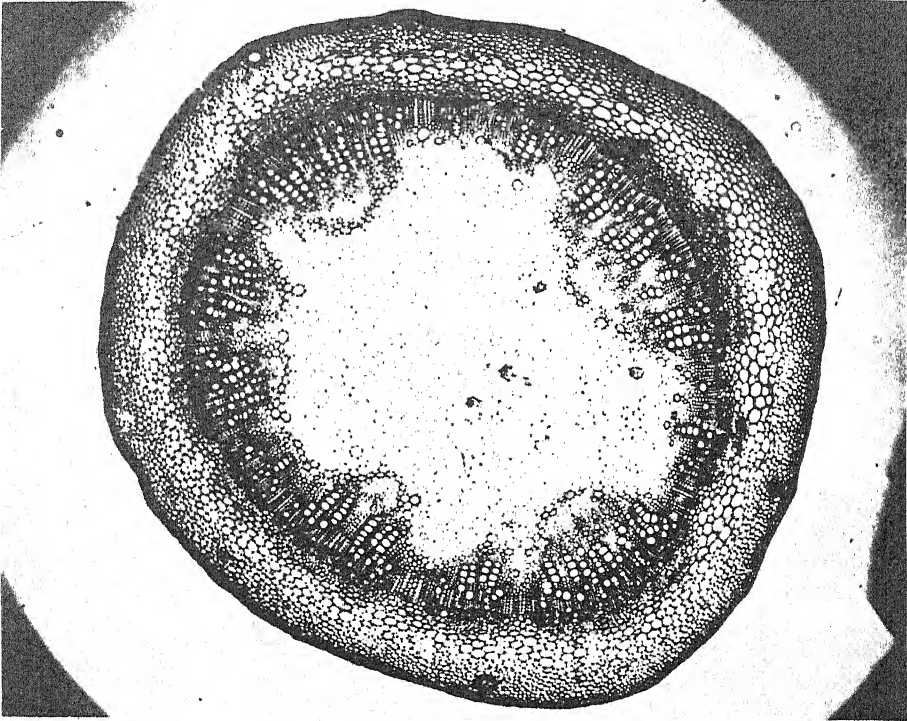
Illustrating Dr. E. Phillis and Dr. T. G. Mason's paper on 'Studies on the Transport of Carbohydrates in the Cotton Plant. III. The Polar Distribution of Sugar in the Foliage Leaf'.

Fig. 1. Transverse section of petiole. × 20.

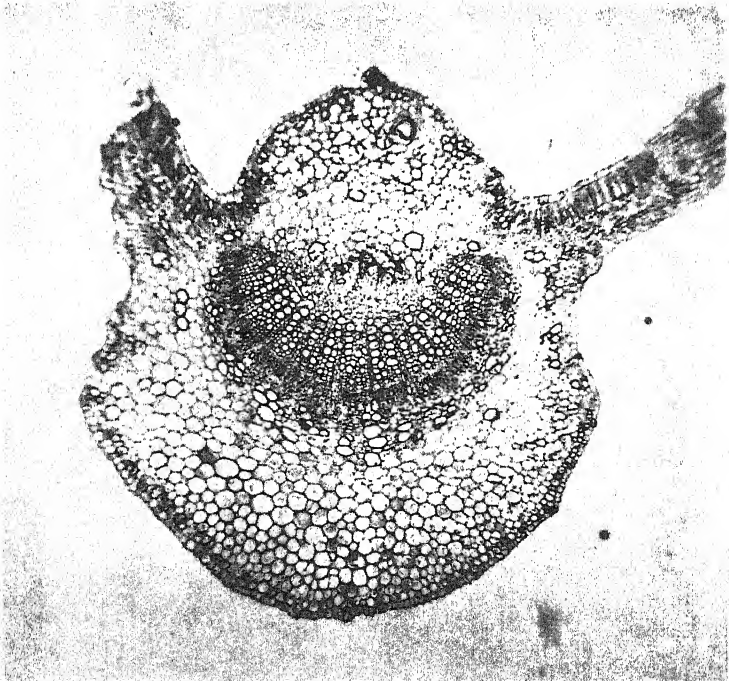
Fig. 2. Transverse section through fine vein. × 1500.

Fig. 3. Transverse section of primary vein. × 30.

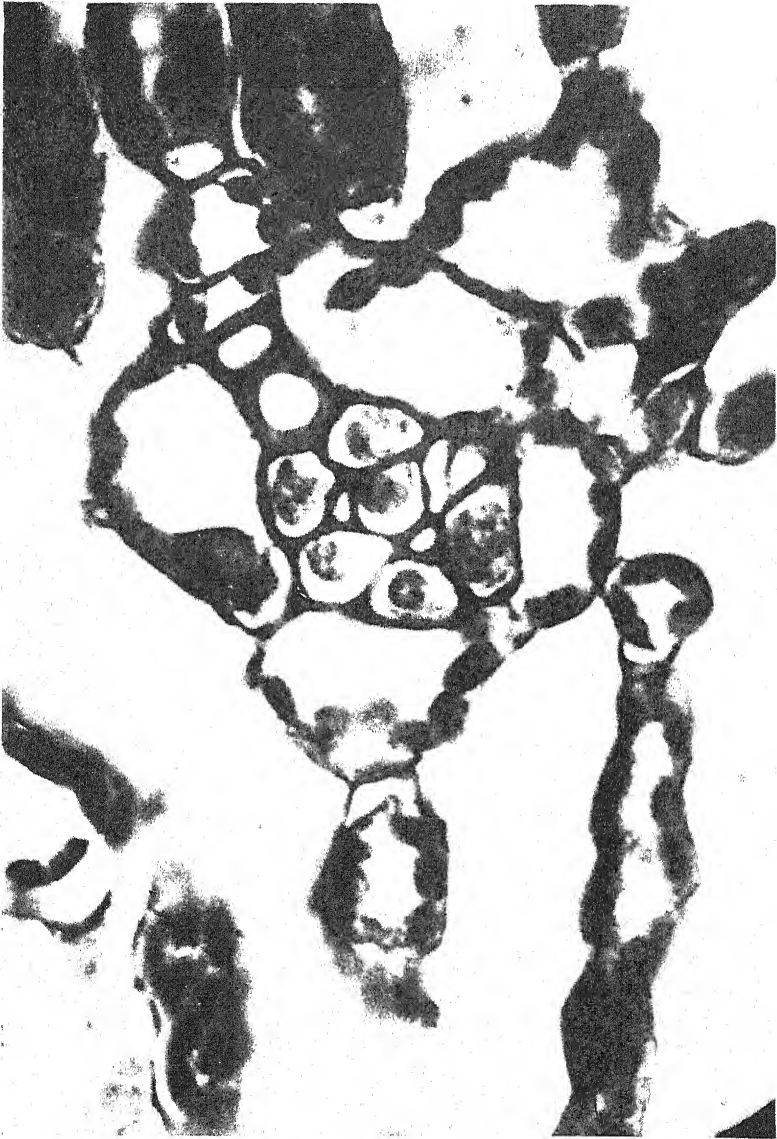




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3







# The Mechanism of Water Conduction in the Musci considered in Relation to Habitat.

## II. Mosses Growing in Damp Situations.

BY

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(Department of Biology, University College of Swansea.)

With forty-one Figures in the Text.

AN investigation of the method of absorption and conduction of water in mosses growing in wet situations (3) showed, that all mosses occupying this type of habitat are able to conduct water when environmental conditions are such that only the basal portions of the stem are in contact with water. The fact that the capacity for conduction varies with the habit and habitat of the plants was also apparent. Further investigation has been made on the absorbing and conducting capacity of mosses growing in a second *damp* type of habitat, where there is less ground-water and consequently where the degree of humidity of the atmosphere is lower. The result of this investigation is the subject matter of the present report.

Of the mosses studied nine species can be said normally to occupy such habitats. These species are *Eurhynchium striatum*, *Porotrichum alopecurum*, *Hylocomium squarrosum*, *Brachythecium purum*, *Hypnum Schreberi*, *Hypnum cupressiforme*, *Climacium dendroides*, *Thuidium tamariscinum* and *Mnium punctatum*.

### I. *Eurhynchium striatum*.

This moss is found growing typically on branches of trees near waterfalls or in the shelter of grasses and larger mosses on the drier banks of bogs and meadows. The individual plants are often twenty centimetres in length, slender and prostrate. The stems are densely and pinnately branched, the branches being more or less ascending. The leaves are crowded and imbricated, widely cordate-triangular, but the insertion at the base is narrow and only slightly decurrent (Fig. 1).

Experiments were carried out to determine the rate of external and

internal conduction in this and all succeeding mosses treated in this communication, precisely according to the methods already described (3).

*Rate of external conduction.*

An investigation of the rate of external conduction of this moss gave the following typical results—Tables I and II.

TABLE I.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	3.08	0.78	0.78	0.95	0.95	0.95	1.15
2. „	5.06	0.38	0.4	0.4	0.4	0.45	0.84
3. (Iron)	2.96	0.62	0.66	0.68	0.74	0.78	0.78
4. „	5.06	0.58	0.58	0.58	0.58	0.58	0.59
5. (Acid)	3.16	2.65	2.79	2.92	2.93	2.96	2.96
6. „	6.55	1.14	1.16	1.24	1.26	1.33	1.99

TABLE II.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	5.2	1 hr.	1.0
2.	9.5	2 hrs.	1.2
3.	5.5	3 hrs.	1.1
4.	3.5	6 hrs.	0.8
5.	6.5	24 hrs.	2.1

The rate of external conduction is evidently very slow, but it must be remembered that the plant in nature is inclined and often quite prostrate. Further experiments made with plants in their naturally inclined position showed that although the rate of conduction is more rapid under these circumstances it is still insufficient to enable water to reach the tips of the plants.

*Amount of external conduction.*

The amount of liquid conducted externally is also very small, as can be seen from Table III.

TABLE III.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	2.4	1 day	0.2
2.	4.0	„	1.08
3.	5.5	5 days	0.35
4.	3.2	„	0.12

The comparatively large amount of water conducted by one plant in one day is apparently due to the densely branched nature of the stem.

*Rate of internal conduction.*

In an investigation of the rate of internal conduction the following results were obtained :

TABLE IV.

Plant number.	Time.	Readings for potassium nitrate in cm.			Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of KNO <sub>3</sub> .	Length of plant.	Region submerged.	Internal rise of Li <sub>2</sub> SO <sub>4</sub> .
1.	1 hr.	4.5	1.0	0.2	5.4	0.7	0.5
2.	2 hrs.	6.4	0.5	0.8	8.2	0.8	0.9
3.	3 hrs.	6.5	0.7	1.0	6.8	0.5	0.6
4.	4 hrs.	10.0	2.0	2.5	5.1	1.0	1.2
5.	18 hrs.	4.5	0.5	0.3	3.8	0.6	0.9
6.	24 hrs.	6.8	0.9	1.4	4.0	0.5	1.6

The rate of internal conduction is again slow, and in no case was there an internal rise of salts for a greater distance than 1.6 cm. in 24 hours. Thus, as for all cases previously reported (3), it is to be expected that in this moss the lack of internal conduction is correlated with a lack of differentiation of internal conducting tissue, and this point was put to the test by microscopic examination.

*Internal anatomy of the stem and leaf of Eurhynchium striatum.*

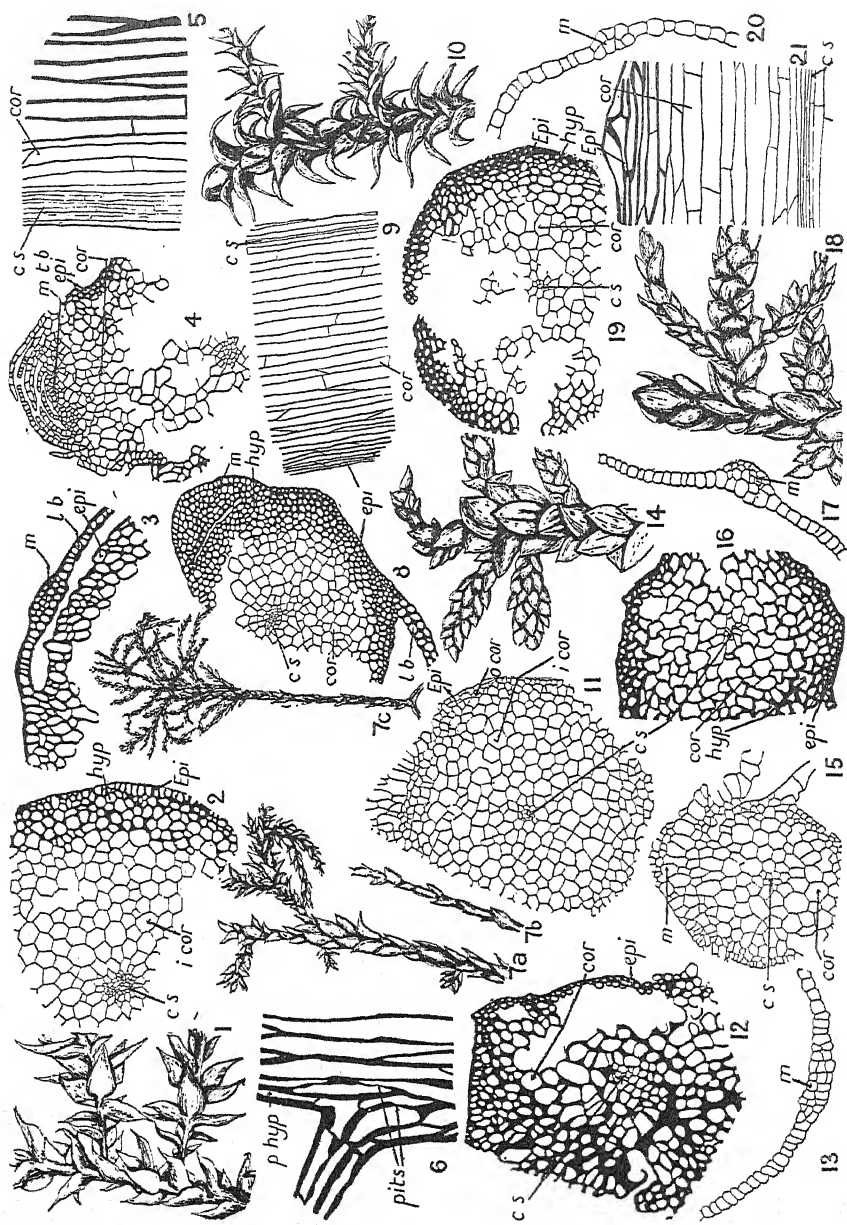
Serial sections of the stem (Figs. 2, 5, and 6), revealed a thin-walled epidermis and outer cortex in the younger portions of the stem, but that these tissues rapidly become thicker-walled and densely pitted as the plant grows older ; an inner cortex of large, unthickened cells, and a relatively large central strand of small, thin-walled cells with definite transverse walls, which occupies approximately one-tenth of the diameter of the transverse section.

The thick-walled epidermis and hypodermis of the older parts of the stem are often interrupted by the origin of numerous branches which are continuous with the cortex of the stem, so that the unthickened central tissue of the branches is continuous with the unthickened cortex of the main stem (Fig. 4).

The leaf lamina is continuous with the thick-walled epidermis of the stem, and the midrib, which consists of from twenty to twenty-five thick-walled cells, as seen in transverse section, is continuous with the hypodermis of the stem (Fig. 3).

*Entry of materials and path of internal conduction.*

Data obtained by dipping the bases of complete plants of *E. striatum* into dishes of potassium nitrate and treating in the usual manner showed that, whereas penetration through the thin-walled epidermis in the upper



FIGS. 1-21.

parts of the stem was rapid, entry through the thickened epidermis and hypodermis was slow. In many cases entry into the cortex did not occur until two or three days had elapsed. Where branches were numerous, however, penetration was more rapid, for the epidermis and hypodermis of the branches are thinner-walled and so allow of a more rapid entry. Internal conduction occurred in both the central strand and cortex though the rate of such conduction was slow. This was probably correlated with the large number of transverse walls in the cells of the central strand.

It can be concluded that in *E. striatum* there is relatively little external or internal water conduction, and for this reason the plants are restricted to a damp, if not wet, environment.

## II. *Porotrichum alopecurum*.

The large size of this moss, with its characteristic tendency to develop branches only towards the apical region, makes the species an outstanding member of the plant societies found growing abundantly on woody slopes. The stems are robust, from three to eight inches long, unbranched at the base, but densely branched near the apex. The leaves on the main stem are broadly-triangular and scale-like, increasing in size towards the apical regions; while the leaves on the branches are narrow, elliptic-oblong, tapering towards the point of insertion and being finely pointed at the apex. All the leaves are strongly nerved to a point just below the tip (Fig. 7).

*Abbreviations for Figures:*—*cor.*, cortex; *o.cor.*, outer cortex; *i.cor.*, inner cortex; *t.w.cor.*, thick-walled cortex; *c.s.*, central strand; *epi.*, epidermis; *l.e. cell*, large epidermal cell; *hyp.*, hypodermis; *p.hyp.*, pitted hypodermis; *hyd.*, hydroids; *i.l.c.*, irregular cells of lamina; *l.b.*, leaf-blade; *l.t.*, leaf trace; *m.*, midrib; *l.c.c.*, large central cells of midrib; *d.m.l.*, double midrib of leaf; *m.t.b.*, meristematic tissue of branch; *par.*, paraphyses; *p.*, pits.

FIGS. 1-21.—1. Portion of the gametophyte of *Eurhynchium striatum* showing the arrangement and divergence of the leaves.  $\times 6$ . 2. Transverse section of the stem of *E. striatum*.  $\times 96$ . 3. Transverse section of the leaf of *E. striatum*.  $\times 70$ . 4. Transverse section of the stem of *E. striatum* cut in the region of a developing branch and showing the continuity of the thin-walled tissue of the stem and branch.  $\times 103$ . 5. Longitudinal section of the stem of *E. striatum*.  $\times 87$ . 6. Longitudinal section of the peripheral tissues of the stem of *E. striatum* showing the pitted hypodermis.  $\times 225$ . 7 a. Portion of the branching region of the gametophyte of *Porotrichum alopecurum* showing the arrangement and divergence of the leaves.  $\times 25$ . b. Portion of the lower region of the stem of *P. alopecurum*.  $\times 25$ . c. Complete gametophytic plant of *P. alopecurum*.  $\frac{2}{3}$  Natural size. 8. Transverse section of the stem and leaf of *P. alopecurum*.  $\times 47$ . 9. Longitudinal section of the stem of *P. alopecurum*.  $\times 70$ . 10. Portion of the gametophyte of *Hylacomium squarrosum* showing the arrangement and divergence of the leaves.  $\times 3$ . 11. Transverse section of a young stem of *H. squarrosum*.  $\times 150$ . 12. Transverse section of an older stem of *H. squarrosum* showing an extreme thickening of the cells of the cortex resulting in the obliteration of the lumen of some of the cells.  $\times 190$ . 13. Transverse section of the leaf of *H. squarrosum*.  $\times 138$ . 14. Portion of the gametophyte of *Brachythecium purum* showing the arrangement and divergence of the leaves.  $\times 37$ . 15. Transverse section of a young stem of *B. purum*.  $\times 120$ . 16. Transverse section of an older stem of *B. purum*.  $\times 103$ . 17. Transverse section of the leaf of *B. purum*.  $\times 70$ . 18. Portion of the gametophyte of *Hypnum Schreberi* showing the arrangement and divergence of the leaves.  $\times 35$ . 19. Transverse section of the stem of *H. Schreberi*.  $\times 103$ . 20. Transverse section of the leaf of *H. Schreberi*.  $\times 103$ . 21. Longitudinal section of the stem of *H. Schreberi*.  $\times 86$ .

*Rate of external conduction.*

Typical results of the rate of external conduction of solutions are given in Tables V and VI.

TABLE V.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	5.35	1.0	1.25	1.3	1.3	1.3	1.5
2. "	7.6	1.55	2.5	2.58	2.6	2.7	2.8
3. (Iron)	6.47	1.43	1.5	1.5	1.5	1.6	1.6
4. "	10.28	1.1	1.3	1.35	1.35	1.4	1.7
5. (Acid)	6.5	3.45	3.8	3.85	3.9	4.0	4.0
6. "	10.7	1.3	1.3	1.35	1.5	1.6	2.0

TABLE VI.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	10.1	1 hr.	1.4
2.	8.7	2 hrs.	3.1
3.	3.5	3 hrs.	2.7
4.	7.3	24 hrs.	2.2
5.	12.2	4 days	2.8

The rate of external conduction is obviously very slow in this form and can be correlated with the sparse arrangement of the scale-like leaves on the stem and the narrow base by which they are attached.

*Amount of external conduction.*

Typical results obtained in an investigation into the amount of liquid conducted externally are given in Table VII.

TABLE VII.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	8.6	1 day	0.29
2.	8.2	"	0.41
3.	7.5	5 days	1.75
4.	6.5	"	0.35

The above figures confirm the conclusion that the amount of external conduction in this moss is extremely small, especially in view of its relatively thick stems.

*Rate of internal conduction.*

An investigation of the internal conducting capacity of this moss gave the following typical results—Table VIII.

TABLE VIII.

Plant number.	Time.	Readings for potassium nitrate in cm.			Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	1 hr.	7.3	0.2	2.4	7.5	0.3	2.0
2.	2 hrs.	6.3	0.4	0.3	7.5	0.6	0.8
3.	3 hrs.	7.5	0.5	1.2	6.8	0.5	1.8
4.	4 hrs.	6.3	0.5	1.8	8.2	0.5	1.4
5.	1 day	6.8	0.2	1.6	5.5	1.0	2.8
6.	2 days	8.3	0.3	2.2	4.5	0.3	1.3

The above figures show that the internal conducting capacity of this moss is also very slow, and in view of the size of the plant, insufficient to supply it with all the water it requires.

*Internal anatomy of the stem and leaf of Porotrichum alopecurum.*

Sections of the stem of this moss showed (Figs. 8 and 9), a small-celled epidermis and an outer cortex which become thick-walled in the older portions; a large, thin-walled, inner cortex and a very limited number of small cells forming the central strand, these cells being short when seen in longitudinal section. In the leaf a single-layered lamina occurs together with a midrib consisting of a large number of undifferentiated cells which are continuous with the hypodermis of the stem (Fig. 8).

*Entry of materials and path of internal conduction.*

Experiments carried out in the usual way with complete plants showed that if liquids reached, or were presented to the apex of this moss, then the penetration of the liquid into the apical tissues was fairly rapid; while in all other regions of the stem penetration was found to be very slow. In view of the slow rate of external and internal conduction, as seen in the above tables, it is obvious that the total water requirements of the plant cannot be derived by upward conduction from the soil in which it grows, but must reach the absorbing apex in some other manner. The habitat of the plant is interesting in this connexion, for it normally grows on sloping and therefore well-drained ground in woods or near waterfalls, and was found by the writer to be most abundant in little hollows below overhanging banks. In such situations it seems most probable that an appreciable quantity of drainage-water from such banks, containing salts in solution, would drip on to the apical regions of the plant, being retained amongst the dense apical leaves and absorbed by the apical tissues. This abundant supply of water and salts probably accounts for the fact that this moss, with no marked power of internal or external conduction, is yet one of the largest of British species.

III. *Hylocomium squarrosum*.

This characteristic moss is found growing very abundantly on mossy banks where the grass and taller vegetation cast a considerable amount of shade. The plants occur quite separately and vary from four to twenty centimetres in length. The lower part of the stem is prostrate and covered by surrounding vegetation, but the upper parts of the stem are erect. The stem is branched, the branches being slender and decurved. The plant is crowded with imbricating leaves which from an erect, sheathing, cordate-ovate base suddenly become squarrose and gradually taper into a long, linear acumen (Fig. 10).

*Rate of external conduction.*

Some typical results obtained for the rate of external conduction are given in Tables IX and X.

TABLE IX.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	2.9	2.0	2.17	2.24	2.45	2.86	2.9
2. "	3.3	0.54	0.69	0.69	0.74	1.3	1.77
3. (Iron)	3.78	1.78	2.05	2.1	2.12	2.5	2.62
4. "	5.09	0.96	1.09	1.29	1.29	1.33	1.46
5. (Acid)	2.29	1.56	1.56	1.59	1.84	1.91	1.95
6. "	4.3	2.62	2.64	2.67	2.7	2.9	2.96

TABLE X.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	5.5	1 hr.	1.9
2.	4.0	2 hrs.	2.5
3.	4.5	3 hrs.	2.9
4.	8.0	6 hrs.	3.0
5.	9.0	24 hrs.	4.0

From the above tables it is evident that while the rate of external conduction here is greater than for the previous mosses examined, it is yet too slow and insufficient to supply water to the higher parts of the stem. Again, however, it must be remembered that the greater part of the stem is prostrate and hidden by the surrounding vegetation, which factors not only prevent the evaporation of water but also facilitates its conduction externally.

*Amount of external conduction.*

An investigation of the amount of water conducted externally gave results of which Table XI is typical.



TABLE XI.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	4.5	1 day	0.38
2.	5.0	"	0.58
3.	3.4	5 days	1.46
4.	7.5	"	3.04

The above table shows that a considerable amount of water is conducted over the external surface, the amount being far greater than for any moss so far examined.

*Rate of internal conduction.*

Attention was now directed towards the investigation of the internal conducting capacity of this moss, and the following typical results were obtained—Table XII.

TABLE XII.

Plant number.	Time.	Readings for potassium nitrate in cm.			Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	1 hr.	2.5	0.3	0.2	7.2	0.4	0.8
2.	2 hrs.	2.5	0.4	0.3	8.1	0.9	0.6
3.	3 hrs.	6.4	0.6	0.5	5.6	0.5	0.9
4.	4 hrs.	4.5	0.4	0.3	9.1	0.8	1.0
5.	18 hrs.	7.2	0.3	0.2	5.8	0.3	1.2
6.	24 hrs.	6.0	1.0	1.6	5.5	0.7	1.1

It is evident that the capacity of this moss for conducting water is negligible, for the table shows a rate even smaller than that described for mosses inhabiting very wet situations.

*Internal anatomy of the stem and leaf of Hylocomium squarrosum.*

Transverse sections taken near the apex of the plant showed, a thin-walled, small-celled epidermis; an outer cortex of thin-walled larger cells; an inner cortex of still larger, thin-walled cells and a central strand of a very few, small, thin-walled cells often only from six to twelve cells in number as seen in transverse section and occupying about one-eighteenth of the diameter of the stem (Fig. 11).

Transverse and longitudinal sections taken further down the stem of the plant showed that in the older portions the epidermis and outer cortex quickly becomes thickened and very sparsely pitted, leaving only the inner cortex and central strand thin-walled. Moreover, in a large number of

cases all the tissues of the stem become thickened to such an extent that the lumina of some cells are completely obliterated (Fig. 12). This thickening usually occurred in the older parts of the stem, but it was frequently found quite near the apex. It seems that this extreme thickening of the cells might account for the slowness of internal conduction, and in such cases the only means the plant has of transporting water is to conduct it externally.

As in all cases so far examined the midrib of the leaf terminates in the outer cortex or hypodermis. In transverse section it is seen to consist of two groups of about fifteen cells each, the groups being separated by a single layer, two or three cells wide (Fig. 13).

*Entry of materials and path of internal conduction.*

Complete plants, the bases of which had been dipping into solutions of potassium nitrate, on sectioning and mounting in diphenylamine showed that where any internal conduction had occurred this conduction took place through the longer cells of the central strand, but in all cases examined the coloration of this tissue occurred after longer periods than were necessary to show coloration in the higher regions of the plant. Where external conduction had taken place as far as the relatively thin-walled epidermis, penetration and coloration of the cortex and central strand was rapid, the colour diffusing more slowly up and down the stem; but where the outer cortex was thickened penetration was slower, and in most cases slight coloration was visible in these plants only after twenty-four hours, whilst complete coloration was present in the upper regions after two hours had elapsed.

Thus in *Hylocomium squarrosum* external conduction is more efficient than in most other forms so far examined, and this efficiency is correlated with the closer imbricating nature of the leaves on the stem and also with the slightly sheathing nature of the leaf base. Internally, however, the plant shows no more differentiation of tissue than in other forms, and its power of internal conduction is small.

#### IV. *Brachythecium purum.*

The habitat of this species is very similar to that already mentioned for *Hylocomium squarrosum*, for it is found very abundantly on the sides of mossy banks in the drier regions of bogs and meadows. It occurs in loose, soft patches—the individual plants being quite free but loosely interwoven with the surrounding vegetation. The stems are robust and tumid, varying from three to twenty centimetres in length. They are often prostrate for the basal two-thirds of their length, the apical one-third being more ascending. The stems are pinnately and densely branched and are crowded with wide, concave leaves, whose bases are not decurrent (Fig. 14).

*Rate of external conduction.*

The rate of external conduction of water by this moss was investigated in the usual way with the following results—Tables XIII and XIV.

TABLE XIII.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	4.73	2.83	2.93	2.95	3.0	3.12	3.12
2. "	3.12	1.63	1.68	1.72	1.75	1.79	1.95
3. (Iron)	3.5	2.98	3.09	3.14	3.18	3.26	3.26
4. "	5.64	2.71	2.71	2.75	2.75	2.75	2.75
5. (Acid)	4.35	1.58	2.58	3.14	3.3	3.46	Tip
6. "	3.12	2.04	2.18	2.62	2.7	Tip	Tip

TABLE XIV.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	11.5	1 hr.	2.5
2.	10.0	2 hrs.	3.6
3.	7.0	3 hrs.	5.5
4.	15.5	6 hrs.	5.2
5.	14.5	24 hrs.	6.4

The above tables show that the rate of external conduction in this moss is rapid, the greatest ascent taking place in the first three hours, and in a few cases the rate was found to be sufficient to supply the tips of the plant with water.

*Amount of external conduction.*

Investigations into the amount of water conducted externally were made and typical results obtained are given in Table XV.

TABLE XV.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	2.9	1 day	0.76
2.	4.0	"	0.4
3.	5.0	5 days	1.85
4.	4.5	"	2.0

The amount of external conduction is evidently quite considerable and it can reasonably be concluded that for a large number of these mosses it is sufficient to maintain the plants in a healthy condition. It should be pointed out that, here again, the moss normally grows in an ascending but not vertical position, and this fact together with the shade and association of other plants will ensure a rate of conduction even greater than that accounted for in the above tables.

*Rate of internal conduction.*

Typical results of the rate of internal conduction are given in Table XVI.

Table XVI.

Plant number.	Time.	Readings for potassium nitrate in cm.			Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	1 hr.	7.5	1.3	1.3	6.8	0.4	0.6
2.	2 hrs.	9.5	1.0	1.1	7.5	1.0	0.8
3.	3 hrs.	10.0	0.6	2.0	5.2	0.6	0.6
4.	4 hrs.	10.0	0.6	0.6	12.6	0.5	0.9
5.	18 hrs.	5.5	0.8	0.8	9.1	0.5	1.3
6.	24 hrs.	6.5	0.7	1.5	8.5	0.8	1.5

Again it is evident that this moss is no more able than the preceding ones to conduct water internally.

*Internal anatomy of the stem and leaf of Brachythecium purum.*

Sections of a young stem showed (Fig. 15), an epidermis of thin-walled cells with slightly protruding cell-walls; an outer cortex of small thin-walled cells; an inner cortex of large unthickened cells and a central strand occupying about one-tenth of the diameter of the stem and composed of small, long, narrow, thin-walled cells.

In older stems (Fig. 16) the epidermis and then the outer cortex soon become thickened and pitted, and even the inner cortex in some cases behaves similarly, so that the whole transverse section of the stem is thickened and affords a more formidable barrier to the entry of materials.

The leaf of *Brachythecium purum* (Fig. 17) consists of a single layer of papillose cells, as seen in transverse section, the papillae protruding towards the stem. The leaf is continuous with the epidermis of the stem, each leaf insertion occupying about one-third of the diameter of the stem, whilst the midrib, which consists of about twenty-five cells in transverse section, is continuous with the hypodermis.

*Entry of materials and path of internal conduction.*

Data obtained in the usual way show that the entry of materials through the hypodermis is very slow, and in many cases is not evident for three days; entry through the tip of the stem and through the points of insertion of the numerous branches, however, is rapid and is often distinguishable in from two to three hours.

It can be concluded, therefore, that *Brachythecium purum* has a very

efficient means of conducting water externally, though the rate and amount of external conduction are not so great as might be expected from the arrangement of the leaves on the stem. It seems probable, however, that the papillose nature of the cells of both the epidermis of the stem and the leaf-blade interferes with the continuity of the capillary films of water over the external surface of the plant. In most of the plants of this species which were examined external conduction is by far the more rapid and efficient means of raising water, while internal conduction through the central strand is slow and never succeeds in raising water above the basal regions of the stem.

### V. *Hypnum Schreberi*.

This moss is found in Gower closely associated with *Brachythecium purum* which it resembles to a very marked degree. The individual plants are long, the basal portion of the stem being more or less prostrate as in *Brachythecium purum*, but the upper parts of the stem are erect and rigid and have a characteristic red colour. The stem, too, is densely and pinnately branched and the branches are frequently crowded near the top of the stem. The leaves are closely arranged, imbricated and erect with sheathing bases (Fig. 18).

#### *Rate of external conduction.*

The external conducting capacity of this moss was measured and results obtained are given in Tables XVII and XVIII.

TABLE XVII.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	4.0	2.95	3.05	3.54	3.62	3.72	3.8
2. „	4.3	3.2	3.52	4.11	4.2	Tip	—
3. Iron	3.0	1.6	2.12	2.51	2.93	2.93	2.93
4. „	4.19	2.2	2.51	2.93	3.01	3.15	3.15
5. (Acid)	5.0	2.96	3.11	3.2	3.32	3.5	3.75
6. „	4.7	2.52	2.63	2.65	2.75	3.0	3.53

TABLE XVIII.

Plant number.	Length of plant in cm.	Time.	External rise of KNO <sub>3</sub> in cm.
1.	7.3	1 hr.	3.8
2.	7.5	2 hrs.	3.4
3.	10.5	3 hrs.	4.2
4.	9.4	6 hrs.	5.8
5.	5.5	24 hrs.	Tip

It is obvious that external conduction in this moss is more rapid than in *Brachythecium purum*, a fact which can be correlated with the closer arrangement of the leaves and with their sheathing bases.

*Amount of external conduction.*

An investigation of the amount of water conducted externally gave results of which Table XIX is typical.

TABLE XIX.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	5.6	1 day	1.55
2.	4.7	"	0.52
3.	6.5	5 days	2.28
4.	3.4	"	1.23

The amount of water conducted externally is large, and this fact again can be attributed to the closer sheathing arrangement of the leaves.

*Rate of internal conduction.*

The rate of internal conduction was measured by the rate of conduction of solutions of potassium nitrate and lithium sulphate with the following results.—Table XX.

TABLE XX.

Plant number.	Readings for potassium nitrate in cm.				Readings for lithium sulphate in cm.		
	Time.	Length of plant.	Region submerged.	Internal rise of KNO <sub>3</sub> .	Length of plant.	Region submerged.	Internal rise of Li <sub>2</sub> SO <sub>4</sub> .
1.	1 hr.	9.5	0.6	0.4	9.3	0.7	0.5
2.	2 hrs.	9.5	0.3	0.3	6.9	0.3	0.6
3.	3 hrs.	10.0	0.6	0.2	4.9	0.9	0.4
4.	4 hrs.	7.5	0.8	0.8	7.4	0.6	0.9
5.	18 hrs.	5.5	0.7	0.8	6.5	0.5	1.0
6.	24 hrs.	7.0	0.8	1.1	7.5	0.8	1.4

The rapid external rise of solutions is here again associated with a slow rate of internal conduction, for only after eighteen hours does the internal rise of liquid exceed 1 cm.

*Internal anatomy of the stem and leaf of Hypnum Schreberi.*

Transverse sections of the stem of this moss showed (Figs. 19 and 21), an epidermis of small cells which are thin-walled at the apex, but which become thickened immediately behind the apex; an outer cortex which again becomes thickened near the apex and which becomes a very thick-walled, sparsely pitted hypodermis in the lower parts of the stem; a thin-walled inner cortex of large cells; a small central strand consisting of from five to eight cells which are long, narrow and unthickened.

It is interesting to note that in this moss the hypodermis is thin-walled only for a maximum distance of 0.5 cm. behind the apex, so that, even in young stems, this tissue is found in a thickened condition. Moreover, the cortex is often thick-walled (Fig. 22). The epidermis and hypodermis are interrupted by the origin of numerous branches which place the thin-walled tissue of the branch in direct continuity with the thinner-walled cells of the inner cortex.

The numerous leaves are continuous with the epidermis and are composed of relatively thick-walled cells, though the leaves at the apex of the plant have their cells thin-walled (Fig. 20). The midrib is thick-walled and consists of from five to seven cells, as seen in transverse section, which terminate in the hypodermis.

*Entry of materials and path of internal conduction.*

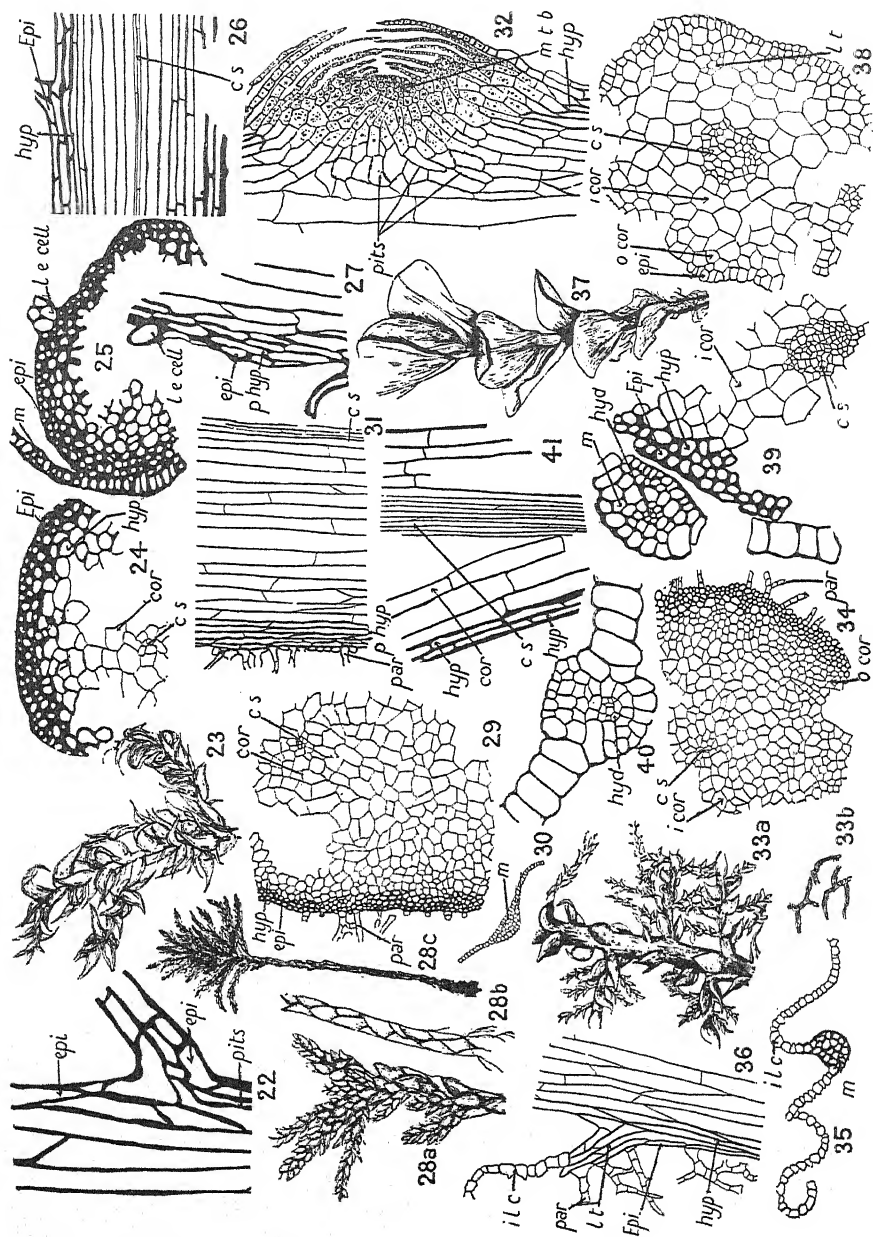
Experiments carried out with complete plants and solutions of potassium nitrate and lithium sulphate showed that the entry of externally conducted solutions is confined largely to the tips of the stem and to the numerous branches, for in many cases no penetration into the internal tissues of the stem had occurred at the end of two days. So constant was this impermeability of the outer tissue found to be, that it is probable that this fact contributes largely towards the success of external conduction in the plant, for the lack of penetration results in the accumulation of water conducted externally in the axils of the sheathing leaf-bases, so that it forms reservoirs from which capillary films can extend to the leaves above. Internal conduction takes place at such a very slow rate through the central tissue that it is evident that the main water supply of this moss is conducted over the external surface to the tip of the stem, where penetration occurs with a later diffusion of this absorbed water throughout the plant.

VI. *Hypnum cupressiforme.*

The habitat of this moss is similar to that of *Brachythecium purum* and *Hypnum Schreberi* and it is frequently found on mossy banks mixed with these two species. The plant appears to grow in loose, intricate tufts, the individual plants varying from two to ten centimetres in length. The stems are slender and clothed with loosely-arranged, imbricating leaves, which are small, ovate-lanceolate, strongly falcato-secund and slightly decurrent (Fig. 23).

*Rate of external conduction.*

The rate of external conduction of this moss was measured and results obtained are given in Tables XXI and XXII.



FIGS. 22-41.



TABLE XXI.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	4.25	1.04	1.41	1.5	1.58	1.65	3.27
2. "	4.75	1.83	1.89	1.95	1.97	2.1	3.55
3. (Iron)	3.96	1.83	1.86	1.88	1.88	1.88	1.9
4. "	3.94	1.61	1.7	1.7	1.7	1.7	1.75
5. (Acid)	5.0	3.64	4.17	4.35	4.35	Tip	—
6. "	3.46	2.63	3.25	3.3	3.37	3.4	Tip

TABLE XXII.

Plant number.	Length of plant in cm.	Time.	External rise of KNO <sub>3</sub> in cm.
1.	5.5	1 hr.	2.7
2.	4.2	2 hrs.	Tip
3.	6.7	3 hrs.	5.1
4.	4.0	6 hrs.	Tip
5.	6.2	24 hrs.	Tip

It is evident that external conduction in this moss is again rapid, for in almost all experiments the liquid was conducted externally to the tip of the plant within twenty-four hours. In others the solutions were conducted to regions near the apex, although the presence of these solutions at the tip was not visible in all cases.

#### *Amount of external conduction.*

Experiments measuring the amount of external conduction were now carried out, and typical results obtained are given in Table XXIII.

The amount of liquid raised over the external surface of this very slender plant is evidently large, and it can be concluded that this plant affords the most successful example of external conduction so far examined.

FIGS. 22-41.—22. Longitudinal section of the peripheral tissues of the stem of *Hypnum Schreberi* showing the sparsely pitted hypodermis.  $\times 226$ . 23. Portion of the gametophyte of *Hypnum cupressiforme* showing the arrangement and divergence of the leaves.  $\times 3$ . 24. Transverse section of the stem of *H. cupressiforme*.  $\times 126$ . 25. Transverse section of the stem and leaf of *H. cupressiforme* cut near the point of insertion of the leaf on the stem.  $\times 110$ . 26. Longitudinal section of the stem of *H. cupressiforme*.  $\times 80$ . 27. Longitudinal section of the peripheral stem tissues of *H. cupressiforme* showing a large-lumened epidermal cell and pitted hypodermis.  $\times 300$ . 28 a. Portion of the apical regions of the gametophyte of *Climacium dendroides* showing the arrangement and divergence of the leaves.  $\times 2$ . b. Portions of the lower regions of the stem of *C. dendroides*.  $\times 2$ . c. Complete gametophytic plant of *C. dendroides*.  $\frac{3}{8}$  Natural size. 29. Transverse section of the stem of *C. dendroides*.  $\times 80$ . 30. Transverse section of the leaf of *C. dendroides*.  $\times 75$ . 31. Longitudinal section of the stem of *C. dendroides*.  $\times 80$ . 32. Longitudinal section of the stem of *C. dendroides* cut at the point of origin of a branch and showing the pitted cortex.  $\times 80$ . 33 a. Portion of the gametophyte of *Thuidium tamariscinum* showing the arrangement and divergence of the leaves of the stem and branches.  $\times 2.6$ . b. Paraphylls of the stem of *T. tamariscinum*.  $\times 55$ . 34. Transverse section of the stem of *T. tamariscinum*.  $\times 90$ . 35. Transverse section of the leaf of *T. tamariscinum*.  $\times 108$ . 36. Longitudinal section of the stem of *T. tamariscinum* showing epidermis, hypodermis, paraphylls, and leaf insertion.  $\times 100$ . 37. Portion of the gametophyte of *Mnium punctatum* showing the arrangement and divergence of the leaves.  $\times 3.7$ . 38. Transverse section of a young stem of *M. punctatum*.  $\times 106$ . 39. Transverse section of an older stem and leaf of *M. punctatum*.  $\times 80$ . 40. Transverse section of the leaf of *M. punctatum*.  $\times 122$ . 41. Longitudinal section of the stem of *M. punctatum*.  $\times 67$ .

The figures given suggest that the rate and amount of this conduction may be sufficient to supply the plant with all its necessary water.

TABLE XXIII.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	4.2	1 day	0.76
2.	5.0	"	0.64
3.	4.5	5 days	1.5
4.	6.5	"	2.9

*Rate of internal conduction.*

With so efficient an external conducting capacity it seemed likely that the rate of internal conduction would be small. This proved to be the case, as can be seen from the data set out in Table XXIV.

TABLE XXIV.

Plant number.	Time.	Readings for potassium nitrate in cm.			Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of KNO <sub>3</sub> .	Length of plant.	Region submerged.	Internal rise of Li <sub>2</sub> SO <sub>4</sub> .
1.	1 hr.	4.5	0.5	0.1	4.6	0.2	0.3
2.	18 hrs.	3.7	0.8	0.2	4.9	0.2	0.3
3.	1 day	3.7	0.5	0.1	6.1	0.5	0.5
4.	3 days	4.5	0.5	0.2	5.3	0.7	0.6

The results show that there is almost an entire absence of internal conduction, for after three days the moss had failed to conduct solutions internally for more than 0.6 cm.

*Internal anatomy of the stem and leaf of Hypnum cupressiforme.*

An examination of serial sections of this moss showed the following tissues (Figs. 24, 26, and 27): An epidermis consisting for the most part of small cells, but interspersed at intervals with large single cells, these larger cells often being three times the diameter of the normal cells. All the cells of the epidermis are thickened to a similar extent in the lower regions of the stem so that the large cells retain a relatively large lumen; an outer cortex of two or four layers of cells which become thickened behind the apex, and are densely pitted; an inner cortex of larger thin-walled cells; a central strand of four or five cells which are long and narrow with few transverse walls.

The leaf is continuous with the epidermis of the stem, whilst the midrib, if present, terminates in the hypodermis, and in transverse section is seen to consist of two small groups of from six to ten thick-walled cells, the groups being separated by a single layer of two or three cells (Fig. 25).



TABLE XXVI.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	7.8	1 hr.	6.3
2.	6.4	2 hrs.	5.1
3.	11.5	3 hrs.	Tip
4.	11.3	"	Tip

It is obvious from the above tables that *Climacium dendroides* possesses the capacity to conduct water to the tip of the plant at a fairly rapid rate.

*Amount of external conduction.*

An investigation into the amount of liquid conducted externally gave the following results: Table XXVII.

TABLE XXVII.

Plant. number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	8.1	1 day	1.93
2.	5.7	"	1.29
3.	6.0	5 days	2.57
4.	6.9	"	2.63

This amount is obviously considerable, and is probably sufficient to supply the plant with all the liquid it requires.

*Rate of internal conduction.*

Typical readings obtained of the rate of internal conduction are given in Table XXVIII.

TABLE XXVIII.

Readings for potassium nitrate in cm.					Readings for lithium sulphate in cm.		
Plant number.	Time.	Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	1 hr.	6.8	0.4	0.9	8.7	0.5	0.6
2.	2 hrs.	7.3	0.6	2.0	6.7	0.6	0.9
3.	3 hrs.	5.5	0.3	0.3	6.4	0.5	2.0
4.	4 hrs.	8.6	0.3	0.7	8.6	0.3	0.8
5.	18 hrs.	7.2	0.6	2.7	5.3	0.4	1.7
6.	24 hrs.	8.7	0.3	2.3	5.6	0.4	1.4

As in the case of most other mosses, it is evident that here the rate of internal conduction is much slower than that of external conduction, and will probably suffice to supply only the lower parts of the plant with water.

*Internal anatomy of the stem and leaf of Climacium dendroides.*

Sections of the stem of this moss (Figs. 29 and 31) showed that, as in other cases, there is little differentiation of the tissues. The central strand consists of only a few small, relatively short cells; the inner cortex of large thin-walled cells; while the outer cortex quickly becomes thickened and pitted. The epidermal cells also are small, and are often modified to form branched, septate hairs.

The midrib of the leaf consists of about twenty-five undifferentiated elements, while the cells of the lamina are irregular and small (Fig. 30).

Sections cut in the region of developing branches (Fig. 32) showed that the cells of the cortex in the vicinity of the branch are very densely pitted.

*Entry of materials and path of internal conduction.*

Experiments carried out with complete plants showed that both penetration at the base and upward conduction are very slow, and that at the end of two days only the basal 2 cm. of the stem contained recognizable amounts of potassium nitrate and eosin respectively. The very rapid external conduction, on the other hand, is to be expected in view of the imbricating arrangement of the leaves on the main stem and branches and the mode of their attachment. Solutions of eosin and potassium nitrate travelling externally reached the apex of most plants in from three to six hours, and penetration of these solutions into all tissues within 1 cm. of the apex was clearly seen at the end of twelve to twenty-four hours. Penetration through the thickened hypodermis was seen to be slow, and conduction through the internal tissues was also found to be slow, a fact which is obviously connected with the lack of internal differentiation of the stem.

VIII. *Thuidium tamariscinum.*

The habitat of this moss is typically that of a shady wood, although it is often found growing on dry mossy banks. The moss is large, being 30 cm. long, and occurs in intricate mats of a bright green colour. The stem is firm, prostrate, and densely branched at intervals. The whole plant is clothed with numerous short paraphylls, but the leaves are less closely arranged than in many previous forms, and do not imbricate. They are broad-based and cordate on the stem, and are smaller, but more numerous on the branches (Fig. 33).

*Rate of external conduction.*

Data obtained for the rate of external conduction are given in Tables XXIX and XXX.

TABLE XXIX.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	3.56	0.98	1.02	1.05	1.15	1.37	Tip
2. "	4.85	1.98	2.1	2.16	3.0	3.16	Tip
3. (Iron)	4.52	2.37	3.21	3.21	3.44	3.95	Tip
4. "	2.58	Tip	—	—	—	—	—
5. (Acid)	2.7	2.51	Tip	—	—	—	—
6. "	4.8	2.6	3.9	3.95	4.2	Tip	—

TABLE XXX.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	6.9	15 mins.	5.2
2.	9.0	30 mins.	Tip
3.	10.5	1 hr.	Tip

This moss shows a more rapid rate of external conduction than any previous form examined. The rapid rate of this conduction can be attributed to the numerous paraphylls, which by their close investment of the stem form narrow capillary channels through which films of water extend between the reservoirs in the leaf axils.

*Amount of external conduction.*

Typical results of the amount of external conduction are given in Table XXXI.

TABLE XXXI.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	4.0	1 day	0.35
2.	4.5	"	0.64
3.	3.4	5 days	1.9
4.	3.2	"	1.4

Although the total amount of liquid raised does not appear to be large, yet in proportion to the size of the plant it is actually quite considerable.

*Rate of internal conduction.*

Table XXXII gives results typical of those obtained for the rate of internal conduction in this moss.

Although in many cases the rate of internal conduction in this moss appears small when compared with the more rapid rise externally, yet in some cases it is obviously important.

TABLE XXXII.

Plant number.	Readings for potassium nitrate in cm.				Readings for lithium sulphate in cm.		
	Time.	Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	1 hr.	7.0	0.5	1.0	8.2	0.5	0.8
2.	2 hrs.	8.0	1.2	1.3	9.2	0.6	1.2
3.	3 hrs.	6.0	0.7	0.8	6.4	0.4	0.8
4.	4 hrs.	8.2	0.6	1.1	9.8	0.5	1.3
5.	18 hrs.	9.5	1.5	1.4	7.3	1.3	2.6
6.	24 hrs.	7.1	1.0	3.0	10.0	0.7	4.7

*Internal anatomy of the stem and leaf of Thuidium tamariscinum.*

Serial sections of the stem were cut, and on examination revealed a thin-walled, ill-defined epidermis which never becomes very thick-walled, and from the surface of which numerous paraphylls arise; a hypodermis of three or four layers of thick-walled, sparsely pitted cells; a cortex of larger, thin-walled cells; a central strand consisting of a few, smaller, thin-walled cells (Figs. 34 and 36).

The leaf lamina is continuous with the epidermis of the stem, the cells being irregular and projecting towards the stem, whilst the midrib terminates in the hypodermis and consists of about twenty thick-walled cells, as seen in transverse section (Fig. 35).

*Entry of materials and path of internal conduction.*

Experiments carried out with complete plants showed that penetration at the tip of the plant is very rapid and often apparent within one hour. Entry is slower in the older regions of the stem, but so rapid is the rate of diffusion in this moss that complete penetration of the liquids used occurs throughout the plant in six hours. Thus it appears that the relatively rapid rate of internal conduction seen in this moss is due, not to the greater elaboration of the central strand, but to the rapid rate of diffusion seen throughout all the plant tissues. Moreover, it seems probable that this rapid rate of diffusion is related to the absence of a thickening of the walls of the epidermis and hypodermis, thus allowing not only a more rapid penetration but also a more rapid transpiration of water, but inducing a higher concentration of osmotic materials in the stem tissues.

IX. *Mnium punctatum.*

This moss inhabits more damp situations than the last five forms studied, for it occurs growing abundantly in damp environments near streams where it forms pure associations of large, dark green tufts. The individual plants are large, often 15 cms. long, the lower parts of the

stem being closely interwoven with tomentum. The stems are firm, erect and sparsely clothed with large, broadly-ovate leaves which, however, become much narrower at the point of attachment to the stem, and are only slightly decurrent (Fig. 37).

*Rate of external conduction.*

The external conducting capacity of the moss was investigated and typical results obtained are given in Tables XXXIII and XXXIV.

TABLE XXXIII.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	2.23	1.46	1.49	1.5	1.5	1.57	1.57
2. "	7.79	1.44	1.45	1.45	1.45	1.45	1.48
3. (Iron)	4.14	0.22	0.23	0.38	0.38	0.38	0.38
4. "	5.83	0.98	1.0	1.04	1.22	1.28	1.37
5. (Acid)	2.65	0.57	0.57	0.57	0.57	0.57	0.86
6. "	3.24	1.29	1.29	1.3	1.34	1.36	1.38

TABLE XXXIV.

Plant number.	Length of plant in cm.	Time.	External rise of KNO <sub>3</sub> in cm.
1.	3.5	1 hr.	2.0
2.	4.0	2 hrs.	2.1
3.	5.0	3 hrs.	2.9
4.	4.2	6 hrs.	2.0
5.	5.5	24 hrs.	3.0

It is evident that while external conduction does take place in this moss the rate is slow. The presence of tomentum, however, ensures that the rate of this conduction is greater than it would have been had the stem been dependent on its scattered leaves with their slightly sheathing bases.

*Amount of external conduction.*

Table XXXV gives typical results obtained in an investigation of the amount of external conduction.

TABLE XXXV.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	1.4	1 day	0.2
2.	2.3	"	0.38
3.	1.9	5 days	0.7
4.	2.2	"	0.42

The amount of liquid conducted externally is obviously very small.



It must be remembered, however, that the plant is normally tufted, and the close contact which it makes with its neighbouring plants in nature would result in a greater rate than is recorded in the experiments with isolated plants.

*Rate of internal conduction.*

An investigation into the rate of internal conduction gave results of which Table XXXVI is typical.

TABLE XXXVI.

Plant number.	Readings for potassium nitrate in cm.				Readings for lithium sulphate in cm.		
	Time.	Length of plant.	Region submerged.	Internal rise of KNO <sub>3</sub> .	Length of plant.	Region submerged.	Internal rise of Li <sub>2</sub> SO <sub>4</sub> .
1.	1 hr.	4.0	0.5	1.2	2.8	0.6	0.9
2.	2 hrs.	4.5	0.6	2.3	6.1	0.6	1.2
3.	3 hrs.	2.7	0.5	Tip	3.6	0.7	1.7
4.	4 hrs.	3.5	0.4	Tip	5.1	0.4	2.9
5.	18 hrs.	5.7	1.2	2.5	3.9	0.5	Tip
6.	24 hrs.	4.6	1.0	Tip	3.4	0.6	2.8

Although the rate of internal conduction is here greater than for any previous moss examined, yet in 24 hours water had risen internally to the tips of very few plants examined.

*Internal anatomy of the stem and leaf of Mnium punctatum.*

Transverse sections of the stem of this moss showed (Figs. 38, 39, and 41), a small-celled, thin-walled epidermis and outer cortex which become thickened and pitted in the older regions of the stem; an inner cortex of larger, thin-walled cells; a central strand of small, long, thin-walled cells. The central strand is comparatively large and occupies from one-seventh to one-fifth of the diameter of the section.

The leaves of *Mnium punctatum* (Fig. 40) are large and consist of a single-layered lamina and a multicellular, several-layered midrib. The midrib consists of about fifty cells, the central six to ten cells of which are small and have been termed 'hydroids'. The midrib terminates in the inner layers of the outer cortex, and the 'hydroids' of the leaf are connected with the central strand only by the cells of the inner cortex.

*Entry of materials and path of internal conduction.*

Investigations carried out with complete plants showed that where these plants were relatively short and tufted, then liquid would rise over the external surface to the unthickened parts of the stem near the apex of the plant. Penetration of liquid in these regions was seen to be rapid. In other plants, however, external conduction was more limited and then

penetration through the thick-walled peripheral tissues was slow, in some cases 24 hours elapsing before the liquids could be demonstrated in the cells of the cortex. It should be stated that cut stems with blocked ends were used in the latter experiments, for it was found that when complete plants were used the rate of internal conduction in the central strand and diffusion of the liquids into the cortex was more rapid than the penetration inwards from externally conducted liquids.

Thus it appears evident that though neither external nor internal conduction is very efficient in this plant, there is a greater differentiation of the central strand than has been observed in the other forms examined with the consequent increase in internal conducting power as compared with preceding forms.

#### CONCLUSION.

From the results recorded in the preceding pages it is evident that the mosses investigated from a damp environment possess the capacity to conduct water. In the great majority of cases external conduction is more rapid than internal, the conducted liquid being absorbed chiefly by the thin-walled tissue at the apices of the stems and branches. In general, the drier the habitat the more marked is the power of external conduction and this is associated with either a more compact arrangement of the leaves, or the presence of numerous paraphylls arising from the epidermis of the stem, both of which result in an increased number of capillary films of water rising up the stem.

The rate and amount of liquid conducted internally was found in most cases to be small, and with this is correlated a lack of differentiation of internal tissues. In the case of the species of *Mnium* investigated, however, the rate of internal conduction often exceeded that of external conduction, and in this moss it was found that there is a greater elaboration of the central strand and of the midrib of the leaf than in the other forms investigated.

#### SUMMARY.

1. Nine species of musci were selected from damp habitats and their method of obtaining water investigated.
2. The external morphology and habit of the plants were studied in connexion with their capacity to conduct water externally, and there was found to be a correlation between the two.
3. The internal structure of the stems and leaves of the species under investigation were examined, and the path of internal conduction and of the entry into the stem tissues of externally conducted liquids were determined.

4. With the sole exception of *Mnium punctatum*, the amount of water conducted over the external surface exceeded that conducted internally.

5. The water conducted externally ascended in the form of capillary films between the leaves and the stem, and was absorbed by the unthickened cells at the apex of the stem and in the leaves and branches, and diffused through the internal tissues in a lateral and downward, rather than in an upward direction.

6. It was found that the water ascending internally travelled through the narrow, elongated, thin-walled cells of the central strand.

7. It was found that, in general, the power of the plant to conduct water both externally and internally diminished as the moisture content of the habitat increased, presumably owing to the fact that the moister the habitat the more water to supply its needs would be deposited from the humid atmosphere over the surface of the plant.

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# On the Conditions Leading to the Injection of Leaves Submerged in Water.

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With two Figures in the Text.

## INTRODUCTION.

THE injection of the intercellular spaces of the leaves of land plants submerged in water is not uncommon. The problem of the mechanism of this injection and the conditions under which it occurs do not, however, appear to have received attention. Dixon (1) has suggested that secretion of water by the mesophyll may play a part even in ordinary transpiration. There is thus the possibility that the injection of submerged leaves may result from the secretion of water into the intercellular spaces.

In 1921 M. J. Gorman, working in this Institute, carried out some observations, the results of which, however, were not published. He showed that excised leaves in a saturated atmosphere showed no injection, those with their blades in the water but their petioles in the air showed only traces of injection, while leaves completely submerged showed injection areas up to one-fourth of their surface. With the cut end of the petiole vaselined and the lamina submerged some injection took place in the regions adjacent to the larger veins, while with the lamina vaselined on both sides and the petiole dipping in water much injection took place.

Gorman also repeated Dixon's (1, p. 23) experiments on the absorption of water by the cut end of leafy shoots completely submerged in water. In some cases there was evidence of absorption, in others not. This discordance can no doubt be explained by differences in the degree of saturation of the shoot, for, according to Smith, Dustman, and Shull (2) the sap-rise in submerged shoots of land plants is due to a saturation deficit in the shoot.

Gorman was inclined to consider injection the result of secretion by the mesophyll cells. It was clear, however, that the problem required further investigation, and a careful series of observations was undertaken in 1932. It may be said at once that the injection of *vaselined* leaves was due to the penetration of that substance into the intercellular spaces. Liquid paraffin is also found to enter the intercellular spaces, and rapidly brings about injection of leaves submerged in it.

#### EXPERIMENTAL TECHNIQUE.

Experiments were conducted with *Eupatorium adenophorum* which has paired leaves. The leaves were removed from the plant by a clean cut at the base of the stalk, tied to glass rods and immersed in water in glass jars, and kept at a constant temperature ( $\pm 0.25^{\circ}\text{C}.$ ) in a water bath. A temperature of  $25^{\circ}\text{C}.$  was found to be suitable, and the majority of the experiments were performed at this temperature. The pairs of leaves were used for these experiments, one of each pair being used as control and the other as the experimental leaf. The amount of injection was graded by eye in categories of percentage of total area. The adequacy of this grading was tested by comparison with the increase in weight of the injected leaves; the data are given later.

Sampling of leaves presented some difficulties, as it was found that the age of the leaves was of importance in determining their behaviour under treatment. Healthy leaves were always used. The entries in the tables represent replicates for each treatment, lowest, middle, and uppermost leaves from the plants being used. All experimental results given in the tables are merely samples from repeated experiments giving similar results.

*Effect of light and darkness.* Two sets of leaves were immersed in tap water in the thermostat in glass jars at  $25^{\circ}\text{C}.$  One of these was completely darkened, the other set was exposed to light from an electric lamp of 150 watts at a distance of 30 cm.

The leaves in darkness began to show injection in five hours, and were completely injected in about fifty hours, after which time some were flaccid and some turgid. The turgid leaves appeared to be uninjured, for by keeping the ends of the petioles in water, and exposing the laminae to air the water in the spaces soon disappeared and left a normal leaf. The flaccid leaves under similar treatment did not recover. After drying the surface, water could be drawn from the intercellular space of such leaves by applying filter paper to the tip of the leaf.

*The illuminated leaves showed no trace of injection, even after 400 hours of continuous exposure.* The water was frequently changed to maintain aerobic conditions. For the first two weeks the leaves remained turgid,

although chlorophyll destruction had begun and the leaves appeared yellow in colour. Some time later the petioles of these leaves were found to be dead and portions of the laminae also, the dead parts appearing as dark injected patches. In order to test whether these leaves were still normal they were now darkened, and complete injection took place in ten hours. On exposure of the leaves to air with the petioles in water the living portions completely recovered. This experiment was repeated several times with similar results. Data of one such experiment are given in Table I. The percentage of area injected was assessed by eye.

TABLE I.

*Injection of Leaves of Eupatorium adenophorum. Effect of Light and Darkness. Unboiled Tap Water. Temp. 25° C. Illumination by 150-watt Lamp at 30 cm.*

Hours.	Percentage of area injected.					
	Dark.			Light.		
	A.	B.	C.	A.	B.	C.
5	trace	trace	trace	0.0	0.0	0.0
13	25	< 25	< 25	0.0	0.0	0.0
24	50	33	> 25	0.0	0.0	0.0
48	100	< 100	90	0.0	0.0	0.0
50		100	> 90	0.0	0.0	0.0
54			100	0.0	0.0	0.0
400				0.0	0.0	0.0
425				25.0	> trace	> trace
				Darkness.		
10				100	100	100

*Mechanism of injection.* These experiments show clearly that illumination prevents the injection which rapidly occurs in the dark, and this has been amply confirmed. Preliminary experiments with daylight had given conflicting results, owing no doubt to the varying intensities employed. It was only after continuous electric light was resorted to that concordant results were obtained.

It would seem clear that, through the photosynthetic process, illumination will keep up the supply of oxygen and so prevent injurious *anaerobic* conditions. The recovery of injected leaves exposed to air is, however, against the suggestion that the injection of darkened leaves is a reaction to injury.

Another effect of carbon-dioxide assimilation in submerged leaves is on the *gas pressure* in the intercellular spaces of the leaf. In the *light* there will be no reduction in the oxygen content of the air of the intercellular spaces of the leaf. In the *dark*, however, the oxygen will be consumed. The corresponding volume of carbon dioxide formed will exert a higher partial pressure than that with which the water is in equilibrium

and will dissolve rapidly in the surrounding water. Since the oxygen has been consumed and not been replaced by another gas, the pressure in the intercellular spaces will fall and water will be drawn in through the stomata.

Consumption of all the oxygen would explain a *twenty per cent.* injection, but in time *complete* injection occurs. A brief consideration will show, however, that this must inevitably follow. When the oxygen is consumed the gas in the intercellular spaces will be roughly *one hundred per cent.* nitrogen, while the surrounding water is in equilibrium with nitrogen at a partial pressure of only four-fifths of an atmosphere. *The nitrogen in the intercellular spaces will therefore dissolve in the water and the spaces become completely injected.*

*Experiments with boiled water.* If the explanation put forward above is the true one, injection should occur rapidly in boiled water. The results of such an experiment are shown in Table II.

TABLE II.  
*Effect of Boiled and Unboiled Water. Leaves in Darkness.*  
*Temp. 25° C.*

Hours.	Boiled water.			Unboiled water.		
	A.	B.	C.	A.	B.	C.
1.0	50	33	> 25	0.0	0.0	0.0
3.0	> 75	75	50	0.0	0.0	0.0
5.0	95	90	75	trace	trace	trace
7.0	100	< 100	85	trace	trace	trace
9.0		100	< 100	25	< 25	< 25
22.0				< 50	> 25	> 25
33.0				75	< 50	< 50
47.0				100	> 75	75
58.0					100	100

It will be seen that injection occurs much more rapidly in boiled than in unboiled water. Injection may be complete in the first at a time when only a trace of injection is visible in unboiled water.

*Effect of light and darkness in boiled water.* The effects of light and darkness on leaves in boiled water at 25° C. was also tested, one set being kept dark, while the other was exposed to an electric lamp (150 watts at 30 cm. distance). The leaves in the light showed as rapid or almost as rapid injection as those in the dark. Such a result is to be expected since the boiled water would tend to dissolve *all the gases* including any oxygen produced in assimilation.

*Effect of access of air to the intercellular space system.* If the injection of the leaves is due to a reduction of gas pressure in the intercellular spaces, access of air to the spaces should prevent injection even if other conditions are favourable to injection. Comparable leaves were placed at 25° C. with



their blades in water and petioles in air. The cut ends of the petioles of one set of leaves were smeared with vaseline and closed rubber tubing tied over them, while the ends of the petioles of the other set were left free and open to air. All the leaves were darkened. The leaves with closed petioles were injected like fully submerged leaves in the dark, whereas those with their ends open to air were not injected even after sixty hours in the dark. These results are shown in Table III.

TABLE III.

*Effect of Free Access of Air to the Intercellular Spaces. Blades of Leaves in Water, Petioles in Air. Darkness, Temp. 25° C.*

Hours.	Percentage of area injected.					
	Petioles closed.			Petioles open to air.		
	A.	B.	C.	A.	B.	C.
10	< 25	trace	trace	0.0	0.0	0.0
24	> 25	< 25	< 25	0.0	0.0	0.0
30	50	25	25	0.0	0.0	0.0
42	100	> 50	50	0.0	0.0	0.0
56		95	75	0.0	0.0	0.0
60		< 100	> 75	0.0	0.0	0.0
75		100	100	0.0	trace	trace

*Demonstration of reduction of pressure in the intercellular spaces of the leaf.* A capillary tube was bent into a U-shape with one limb short and the other long (Fig. 1). Water slightly coloured with eosin was placed in the long arm, leaving a considerable air space in the shorter limb. To the end of the shorter limb a leaf was attached by means of rubber valve-tubing and paraffin wax, so that the intercellular spaces of the leaf were in communication with the air in the short limb of the manometer. The level of the water column in the manometer was marked. The whole apparatus was then kept in darkness at 25° C. with the leaf at the end of its shorter limb under water and the longer limb open to air. After a few hours it was noticed that the water column in the short limb was rising and in about twenty hours had nearly reached the petiole of the leaf. Later the air completely disappeared from the shorter limb of the manometer, and traces of injection were noted. After another sixteen hours the leaf was found to be injected over 75 per cent. of its area, and the water column in the longer limb of the manometer was now rising. Water was entering the leaf, and was passing downwards through the petiole into the shorter limb, as was seen from the dilution of the eosin solution. Eventually the water in the long limb reached the level of that of the water bath, showing that complete connexion had been established through the leaf.

To eliminate the pressure changes due to change of water level in the manometer, the experimental procedure was varied slightly by the use of

the device shown in Fig. 2. To the bottom of a 'boiling' tube was attached a piece of capillary tube (E) with a side arm (C). To the side arm was fixed a piece of rubber tubing, so that it could be closed with a screw clip.

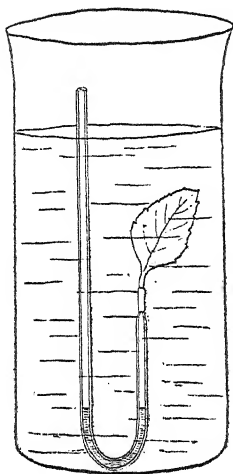


FIG. 1.

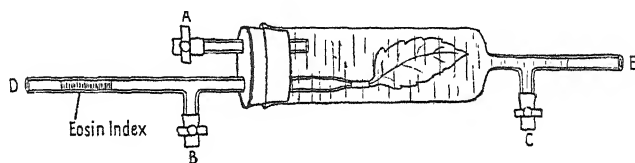


FIG. 2.

FIGS. 1 and 2. FIG. 1. Method of demonstrating reduction of pressure in the intercellular spaces of a submerged leaf. FIG. 2. Apparatus to demonstrate reduction of pressure in a submerged leaf.

The mouth of the tube was fitted with a two-holed rubber-cork, carrying in one of its holes a capillary tube (D) also provided with a side arm opening above the cork (B). Through the other hole of the cork passed a short piece of glass tubing (A). To the end of the capillary tubing (D) a leaf was attached by means of rubber tubing and paraffin wax, so that the intercellular spaces were in communication with the tubing through the petiole. The leaf was immersed in water in the 'boiling' tube which was completely filled with water. By means of the opening (A) and the side arm of the capillary (C) any bubbles in the water were removed, and any pressure on the leaf avoided during the process of fitting the cork. Both these openings were then closed by means of rubber tubing and screw clips. In the first experiment a column of water coloured with eosin, to act as an index, was introduced into the open end of the capillary tube (D) holding the leaf. The opening (B) was completely closed so that the intercellular spaces of the leaf were connected only with air within the capillary tube below the water column. In the second case there was no eosin solution, and the intercellular spaces were directly open to the atmosphere through the capillary tube (D). The leaves in both cases were darkened.

After two hours the eosin water column was found to have travelled towards the leaf, but no injection had occurred. After an interval of fifteen

hours the capillary index had reached the leaf, which was then injected to about 25 per cent. of its entire area. Later it was found that the leaf was fully injected, and the capillary full of water which had passed through the leaf. In the second case there was no injection.

*Path of entry of water into the leaf.* The relative amounts of water entering through the stomata and the petiole of leaves submerged in water were determined in the following manner. Similar leaves were plunged in water, the water removed with filter paper, and the leaves weighed. The cut ends of the petioles of one set of leaves were closed with vaselined rubber tubing, while another set was untreated. The leaves were then completely immersed in water in the dark at three different temperatures 13-14° C., 20° C., and 25° C. After injection had appeared the leaves were removed, dried with filter paper, and weighed again. The results are seen in Table IV below.

TABLE IV.

*Weight Increase (per cent.) after Injection. Leaves Completely Submerged Petioles Closed and Open. Darkness.*

Temperature.	Hours.	Percentage increase.	
		Petioles open.	Petioles closed.
13-14° C.	128.0	19.1	16.1
	128.0	17.5	17.2
	128.0	9.9	8.4
	90.0	20.7	17.5
	90.0	18.7	20.0
20° C.	28.0	11.4	12.0
	28.0	12.6	12.3
	17.5	9.0	8.6
	18.0	8.0	10.5
	18.0	8.2	6.2
25° C.	26.0	8.0	10.0
	47.0	11.2	14.2
	37.5	20.9	22.0
	37.5	14.2	19.1
	40.0	21.4	22.0

The results show that there is little difference in percentage increase of weight between the leaves with open and those with closed petioles. Water apparently enters sufficiently readily through the closed stomata to replace the dissolved gases, so that the extra path through the petiole has little effect. The rate of entry is greatest at 20° C., and is least at the lowest temperature. The temperature optimum may be due to the opposite effects of temperature on rate of gaseous diffusion and on the solubility of the gases.

*Relationship between amount of visible injection and increase of weight.* To test the accuracy with which the amount of water passing into the leaf

can be assessed by eye, a determination was made of the increase in weight of a number of leaves showing various percentages of injection after immersion in unboiled water for various periods. The means, with their standard errors, are shown below (Table V), and indicate that the 'percentage area' method is satisfactory.

TABLE V.

*Relationship between Area of Leaf Injected and Increase in Weight.*

Percentage area injected.	Mean percentage increase in weight.
0.0	$4.02 \pm 0.67$
1-25	$7.92 \pm 0.05$
26-50	$10.83 \pm 0.67$
51-75	$12.50 \pm 0.46$
76-100	$18.00 \pm 0.87$

*Effect of various gases on the rate of injection.* Comparable leaves were submerged in the dark at 25° C. in three vessels of water through which there was bubbled respectively nitrogen, oxygen, and nitrogen plus oxygen. The rate of injection is shown in Table VI below.

TABLE VI.

*Effect of Various Gases. Darkness, Temp. 25° C.*

Hours.	Percentage of area injected.							
	Nitrogen.		Oxygen.		Nitrogen + oxygen.			
	A.	B.	A.	B.	A.	B.	C.	D.
4	0.0	0.0	> 50	50	> 25	50	> 25	25
10	trace	trace	100	100	< 50	> 50	50	< 50
25	{ (pale) trace }	{ (pale) trace }			< 75	> 75	> 75	> 50
4	{ (flaccid) trace }	{ (flaccid) trace }			100	100	100	100

In the nitrogen stream the leaves after some hours were killed as a result of deprivation of oxygen. As a consequence of death respiration ceased and so the absorption of oxygen from the intercellular spaces stopped. Injection therefore occurred only to a small extent.

In the oxygen stream injection occurred rapidly, i.e. after ten hours, since aerobic respiration could continue actively owing to the plentiful supply of oxygen. Furthermore, the oxygen stream would sweep out the nitrogen dissolved in the water, and so bring about the solution of the nitrogen in the intercellular spaces. The diffusion into the intercellular spaces of oxygen from water saturated with that gas seems to be too slow to prevent injection.

With the mixed stream of oxygen and nitrogen, the exact composition of which was not determined, injection was much slower than in oxygen.

Such a mixture of gases would be much more similar to air than a single gas like oxygen, and there would be less sweeping out of one gas by another; on the other hand, respiration would continue and injection ultimately be brought about. The behaviour in the three gases is thus in agreement with the explanation that injection is due to a reduction in the pressure of the gases in the intercellular spaces of the leaf, brought about by consumption and solution of the gases.

We have to thank Dr. F. G. Gregory of this Institute for assistance in the experimental work.

#### SUMMARY.

Injection with water of the intercellular spaces of the leaves of land plants *submerged* in water occurs in darkness but not in the light.

The injection is completely explained by the gas relations of the darkened leaf. The oxygen of the air in the intercellular spaces is consumed in respiration, while the carbon dioxide produced mostly dissolves in the water. As a result the *gas pressure in the spaces falls*, and water is drawn through the stomata into the leaf spaces, and partial injection occurs.

With the removal of the oxygen the concentration of *nitrogen* in the spaces increases above that of the air. The partial pressure of nitrogen is thus greater than that with which the surrounding water is in equilibrium. The gas, therefore, wholly dissolves, and complete injection occurs.

This explanation is supported by a study of the effect on injection of light and darkness, of boiled and unboiled water, and of water through which oxygen, nitrogen, and a mixture of the two gases is bubbled. It is also supported by a study of the pressure changes occurring in the intercellular spaces of leaves submerged in the dark.

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# The Influence of Environment on the Growth and Metabolism of the Tomato Plant.

## I. Methods, Technique, and Preliminary Results.

BY

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With fourteen Figures in the Text.

IN the spring and summer of 1929 and 1930 a considerable amount of work on the effect of the artificial enrichment of greenhouse air with carbon dioxide was carried out by one of the authors. This was continued in 1931 by the authors working conjointly. Increase in fruit yield was obtained from tomato plants, but the conditions necessary for the maximum return for the carbon dioxide used remained obscure. It was decided that as a preliminary a general investigation of the conditions affecting the growth and metabolism of the tomato plant under the ordinary cultural conditions should be undertaken.

The work described in this paper has been devoted to the development of methods for the study of plants growing under the cultural conditions of commerce. Tomato seed of the variety E. S. 1 is sown in boxes of sterilized soil, and the plants are grown to a height of about 1 in. The seedlings are potted singly in '60' pots, and used for experimental purposes when about 6 to 8 in. high and with eight expanded leaves. The time taken for the plants to reach this stage depends upon the season and varies from six to twelve weeks from the time of sowing.

### *Outline of Technique.*

For the study of assimilation, respiration, translocation, and leaf expansion two similar groups of potted plants are used, one group being sampled at the beginning of the experiment while the other is placed in the environment, the effects of which are under investigation. At the end

of the experiment the second group is sampled. Observations made on each group include:—Leaf area, wet weight of leaves, wet weight of stems, wet weight of roots, dry weight of leaves, dry weight of stems, dry weight of roots.

#### *Grading and sampling.*

Some form of grading was found desirable. A system was devised by which the variability of the sample was much reduced. The plants from one sowing of seed were arranged in groups according to height, the height range of each group being 1 in. All except the numerically largest of these groups were rejected. Plants were then selected from this group; being chosen for uniformity on a basis of stem thickness and leaf area as determined by inspection. The reselected group was divided into two equal parts at random, one part being used for the sample at the beginning of the experiment and the other for the sample at the end.

This method is open to the objection that a true sample of the population is not obtained and that full advantage is not taken of the available material. The method was used in the earlier work, but later a much improved method was evolved in which the plants were used in pairs. In the 'paired plant' method a considerable number of healthy plants were taken from one sowing, but without regard to height, stem thickness, or leaf area. These plants were paired by inspection, the factors height, stem thickness, and leaf area being taken into account. One plant, chosen at random from each pair, was sampled at the beginning of the experiment, its partner being sampled at the end. The percentage change of each plant in relation to its partner was calculated and the significance of the mean per cent. change estimated, Fisher's modification of 'Student's method' being used.

#### *Practical requirements.*

The success of the experimental work was found to depend on attention to comparatively small details of technique, particularly in relation to drying and weighing of material, removal of soil from roots, and measurement of leaf area. These will be considered separately.

#### *Drying and weighing.*

A cast iron pot mounted on its side is used as a drying oven. It has a capacity of 16 litres, and an air-tight door (not shown in figure) is fitted. The oven is heated externally by four 36 ohm resistance mats in series and internally by four similar mats, the two series of four are connected in parallel. Each of the four internal mats is mounted between two thick aluminium plates and insulated by means of an asbestos net from the metal. Four shelves are thus produced, each uniformly heated. Each shelf and the oven system as a whole is earthed as a safety measure.



Current consumption when the heating circuit is in operation is about 800 watts, the heating circuit is closed for only a fraction of the drying time so that the mean current consumption is less than 300 watts.

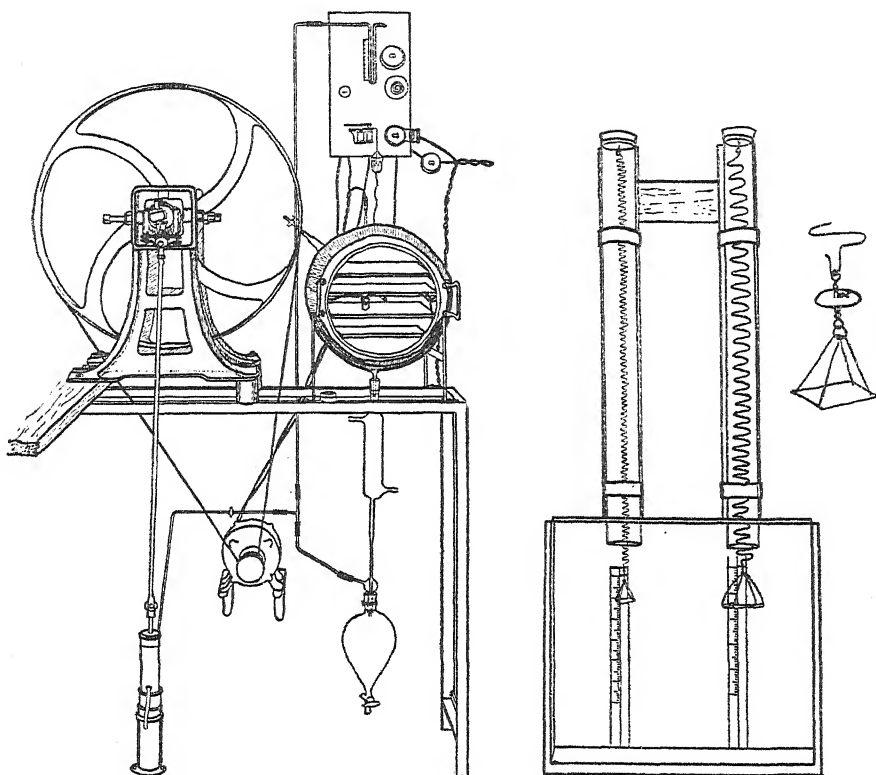


FIG. 1.

FIG. 2.

FIGS. 1 and 2. Fig. 1. Diagram showing electric drying oven with pump and condensing apparatus. Fig. 2. Diagram of Joly balances to show enclosure within glass tubes.

Oven temperature is controlled by a mercury regulator and there is a thermal fuse inside the oven to guard against injury to the apparatus in the event of a failure of the thermostat system.

A hole at the lowest point of the oven connects with a water-cooled double surface condenser and the receiver.

Atmospheric pressure is reduced during drying by an exhaust pump of the 'Geryk' type driven by an electric motor, but the oil in the pump is replaced by glycerine to avoid emulsification by any water reaching it. No hygroscopic drying agent is used. The working temperature of the oven, determined at the surface of the shelves, is  $70^{\circ}\text{C} \pm 5^{\circ}\text{C}$ . Fig. 1 shows the complete oven.

Plant material is contained in perforated zinc trays resting on the

shelves, and drying is carried out at a pressure equivalent to 20 to 30 mm. of mercury as shown by a manometer attached to the oven. To hasten

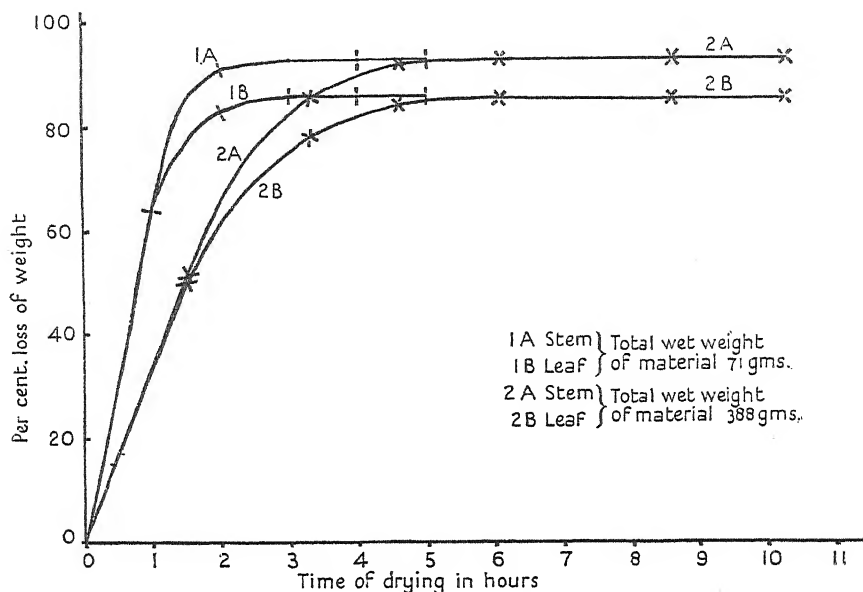


FIG. 3. Graph showing effect of mass of material on time of drying of stems and leaves.

drying the stems and roots are split longitudinally, thus facilitating the escape of water vapour.

Weighing of the dry material is carried out on specially constructed spring balances of the Joly type (Fig. 2), giving weighings with an error of less than 1 per cent. The springs are made of phosphor bronze wire and the whole system is enclosed to avoid air currents. A mirror and special disc indicator are used to avoid errors due to parallax.

The dry material is kept in the hot oven until a few seconds before weighing. For weighing it is placed directly on the scale pan and the weight read at once. No errors within the limit of weighing appear to be introduced by the slight warmth of the material. If on the other hand the whole of the material is removed before weighing is begun, considerable errors are introduced by the absorption of water, the use of an ordinary desiccator being impracticable. Drying under reduced pressure gave more rapid and satisfactory results than drying in an air current. The time taken to attain constant weight depends on the amount of material, but is always less than 7 hours. As a precautionary measure, all material was dried for a standard time of 17 hours. Curves of drying for different weights of material are given in Fig. 3.

Respiration losses during drying have been found to be less than 1 per

cent. of the dry weight of the material. For this determination a method was evolved in which all the carbon dioxide from the oven was absorbed on moist sodium hydroxide and estimated by double titration. The accuracy of the method was tested by introducing known amounts of carbon dioxide.

#### *Measurement of leaf area.*

A rapid and reasonably accurate method has been evolved. It depends on the amount of light intercepted by the leaves under standardized condition; and this, corrected for the transmission factor, provides a means of determining the area. The accuracy of the method is within about  $\pm 2.5$  per cent.

The device which we have called a *phyllometer*, consists of two cylindrical sheet iron drums A and B (Fig. 4). Each is closed at one end, the open ends being hinged together. The dimensions are diameter 11 in. and length 17 in.

The inside of each drum is painted with a commercial preparation of zinc oxide and a final coating of zinc oxide in gelatine is given. The upper drum (A) contains a 60 watt 'pearl' gas-filled electric lamp, a concave disc of opal glass (not shown in figure) being mounted beneath the lamp to act as a partial diffuser. Additional diffusion of the light is obtained by a septum of parchment paper (D) mounted between two glass plates 4 in. from the open lower end of the cylinder. Provision is made for the escape of heat from the lamp. The lower drum (B) is covered at its upper end by a 'leaf plate' (E) composed of two parchment paper septa separated from each other by a sheet of glass and protected by sealed glass coverings. About the mid point of the lower cylinder (B) is fitted an optical device (C) by which the intensity of illumination within the cylinder may be measured. This device is shown in greater detail in Fig. 5.

In use, leaves are placed on the leaf plate (E) and covered by another glass plate to keep them flat, crushing being prevented by small distance pieces. Observations of light intensity on the wall of the lower drum are made with the upper drum in the vertical position. The area corresponding to this intensity is found by reference to a table and correction made for the light transmission of the leaves. This remains fairly constant for leaves of one type but is determined at frequent intervals. The transmission factor also varies according to the position of the leaves on the

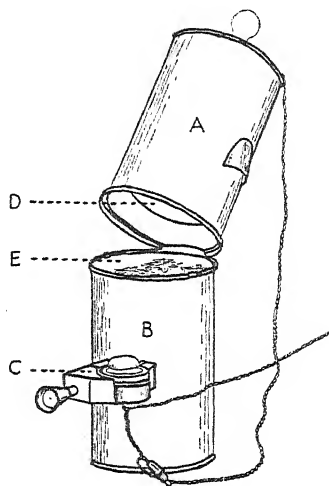


FIG. 4. Phyllometer i.e. apparatus to determine leaf area.

plant, but as all the leaves on each plant are used a mean value is obtained which is satisfactory for the present purpose.

The device seen at C in Fig. 4 is the photometric part of the phyllometer, and is shown in Fig. 5 with the lids removed. In the wall of B is

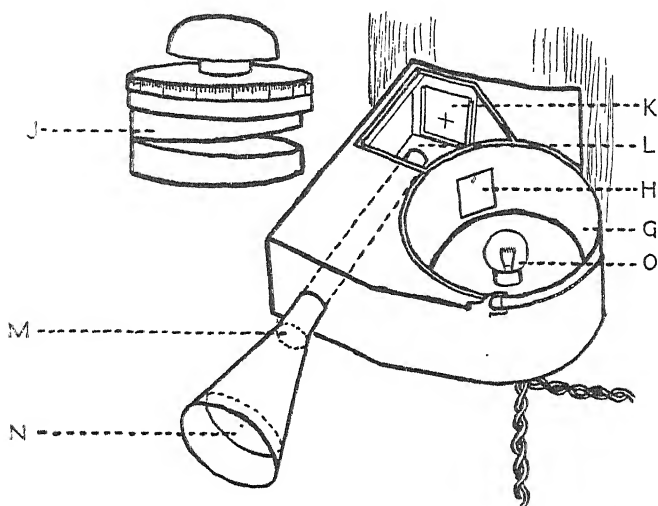


FIG. 5. Details of phyllometer shown in Fig. 4.

a glass window (K) coated with zinc oxide, white, with the exception of a central cross which is left clear. The surface of this window is illuminated through a translucent screen (H) in the wall of the lamp house (G). The amount of light is controlled by a wedge (J), which is attached to the lid of the lamp house. This lid also carries a scale. Matching is obtained by rotating the lid until the visibility of the cross is at a minimum.

The chamber (L) is coated with white and covered by a light-tight lid. At M is a lens focused on the cross and an Ilford 'Gamma' light filter. These have been found to facilitate matching. At N is a metal disc perforated with a pin-hole, observation through this having been found to reduce errors. The inside of the lamp house and the wedge are painted white.

The lamp O (12 volt, 6 watt, gas-filled) is connected in series with the lamp in the drum A, while another 75 watt lamp (P) Fig. 4, in parallel with the lamp in A serves to give the correct amount of current to the 6 watt lamp. Under these conditions the whole circuit is worked from the main supply and an artificially produced variation of 25 per cent. in the voltage of the supply has not been found to affect matching. In practice, voltage variations do not exceed 5 per cent. A calibration curve for the device, obtained by using known areas of cardboard, is shown in Fig. 6.

*Removal of soil from roots.*

Rapid and satisfactory washing of the roots has been attained in the following manner. The stem of the plant is cut off at the cotyledon scars and the soil knocked out of the pot leaving the earth attached to the roots.

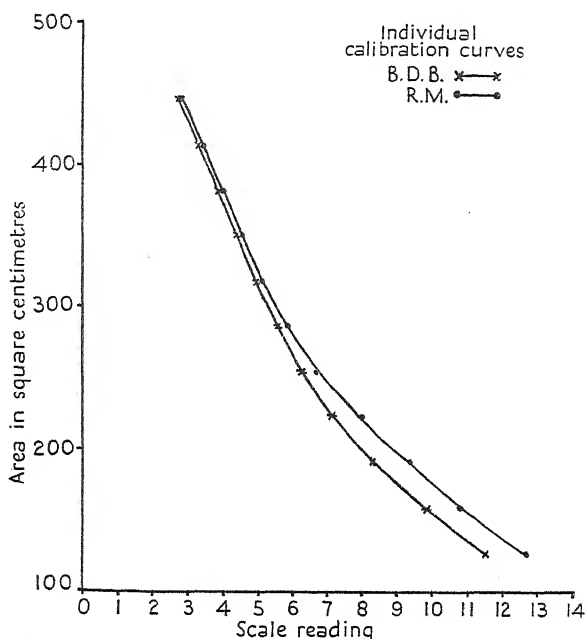


FIG. 6. Calibration curves of phyllometer for two observers.

This mass of earth is immersed in water for not less than five minutes and the greater proportion of the earth then removed by gentle manipulation. The root with the remaining earth is fastened in a spring clip (A, Fig. 7) protected with rubber to avoid damage to the root. The clip forms part of the lid of a metal vessel containing water. Seven such vessels and two lids with clips are shown in the figure. The seven vessels containing the roots and water are shaken simultaneously for two minutes, the frequency of the movement being about 500 per minute and the amplitude about  $\frac{1}{2}$  inch. The root is removed from the clip, rinsed under the tap, and blotted dry. By collecting the fibre broken off it was found that the loss of fibre by this method is of the order of 3 per cent. in the case of healthy roots.

The washing device is shown in Fig. 7. The seven root vessels are fastened on a tinplate base (B), which fits into the container (C). In use they are held in position by a clamp. The vessels are shaken by an eccentric (D), driven by an electric motor (E).

*Measurement of light intensity.*

Several experimental devices are under construction. In the most promising of these incident light is integrated in a special device and allowed to act on a panchromatic photographic plate for a standardized

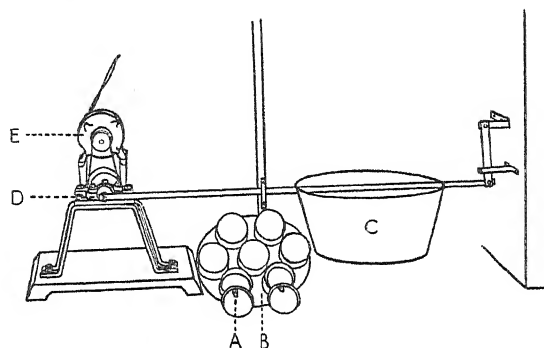


FIG. 7. Shaking apparatus for removal of earth from roots.

period of half a minute. A compensating filter and a neutral wedge are used. The plate is developed under standardized conditions, and a fairly simple relation is found to exist between the amount of light and the density of the image.

The present work has been carried out, using a Holophane Lumeter. Light readings are taken at approximately hourly intervals and a mean determined from which the total light during the 7-hour period is calculated.

*Evaporating power of the air.*

In some experiments determination of the evaporating power of the air was made, using a modified form of Knight's filter paper disc atmometer.

## PRELIMINARY EXPERIMENTAL RESULTS,

A number of experiments have been carried out, and although the data have not yet been fully examined, a number of interesting observations have been made. Some of the results are given below to indicate the scope of the methods and as an introduction to the more detailed study of the metabolism of the tomato plant under cultural conditions.

In the tables below the figure for light is the total in foot-candle hours during the assimilation period, which in all cases is 7 hours. Respiration experiments had a duration of 17 hours. The temperature is the mean temperature as obtained from hourly observations. Where 'atmo-

meter' is given the figures are the mean rate of loss in grams of water per hour from a saturated disc of filter paper 9 cm. in diameter. The probability of the result being due to chance is calculated according to Fisher's modification of 'Student's Method.  $N$  is the number of plants in the first sample and  $n$  is the number of plants in the second sample.

Experiment 13. 22. 8. 32. *Assimilation (7 hours' light).*

Light 3838 f.c.h. Temp. 20.2° C.  $N = 10$ .  $n = 10$ .

	Increase or decrease.	Percentage change.	Probability.
Leaf area . . . .	+ 8.6 cm. <sup>2</sup>	+ 2.47	0.4
Dry weight, leaf . .	+ 0.148 grm.	+ 27.78	< 0.01
„ plant . . . .	+ 0.208 grm.	+ 21.95	0.01
% water, leaf . . .	- 1.70	- 1.86	< 0.01
„ plant . . . .	- 1.00	- 1.07	< 0.01

Experiment 14. 24. 8. 32. *Respiration and Translocation*

(17 hours' darkness). Temp. 17.9° C.  $N = 6$ .  $n = 6$ .

Leaf area . . . .	+ 19.1 cm. <sup>2</sup>	+ 4.95	0.1-0.05
Dry weight, leaf . .	- 0.109 grm.	- 10.28	0.01
„ plant . . . .	- 0.080 grm.	- 4.64	0.4
% water, leaf . . .	+ 2.00	+ 2.26	< 0.01
„ plant . . . .	+ 0.50	+ 0.54	0.02-0.05

Experiment 15. 31. 8. 32. *Assimilation (7 hours' light).*

Light 5453 f.c.h. Temp. 23.5° C.  $N = 7$ .  $n = 7$ .

Leaf area . . . .	- 1.00 cm. <sup>2</sup>	- 0.30	—
Dry weight, leaf . .	+ 0.110 grm.	+ 16.45	0.01-0.02
„ plant . . . .	+ 0.142 grm.	+ 16.02	0.10-0.05
% water, leaf . . .	- 1.628	- 1.80	< 0.01
„ plant . . . .	- 0.71	- 0.76	< 0.01

Experiment 16 A. 19. 9. 32. *Assimilation (7 hours' light).*

Light 1895 f.c.h. Temp. 15.0° C.  $N = 10$ .  $n = 10$ . Atmometer 0.592.

Leaf area . . . .	+ 10.10 cm. <sup>2</sup>	+ 3.30	< 0.01
Dry weight, leaf . .	+ 0.096 grm.	+ 15.56	< 0.01
„ plant . . . .	+ 0.123 grm.	+ 12.02	< 0.01
% water, leaf . . .	- 0.890	- 0.99	< 0.01
„ plant . . . .	- 0.416	- 0.45	0.02-0.05

Experiment 16 B. 20. 9. 32. *Respiration and Translocation (17 hours' darkness).* Temp. 10.0° C.  $N = 10$ .  $n = 10$ . Atmometer 0.071.

Leaf area . . . .	+ 0.70 cm. <sup>2</sup>	+ 0.22	—
Dry weight leaf . .	- 0.056 grm.	- 7.86	0.10-0.05
„ plant . . . .	- 0.033 grm.	- 2.92	0.50-0.40
% water, leaf . . .	+ 0.688	+ 0.77	< 0.01
„ plant . . . .	+ 0.240	+ 0.26	0.10-0.05

Experiment 17. 21. 9. 32. *Assimilation (7 hours' light)*. Light 5687 f.c.h.  
Temp. 20.5° C.  $N = 10$ .  $n = 9$ . Atmometer 0.842.

	Increase or decrease.	Percentage change.	Probability.
Leaf area . . . .	+ 3.10 cm. <sup>2</sup>	+ 1.07	0.20-0.10
Dry weight, leaf . .	+ 0.081 grm.	+ 14.52	0.05-0.02
„ plant . . . .	+ 0.103 grm.	+ 11.15	< 0.01
% water, leaf . . .	- 1.084	- 1.19	< 0.01
„ plant . . . .	- 0.604	- 0.65	< 0.01

The 'paired plant' method was introduced into all experiments subsequent to No. 17, consequently the values given in the tables under 'Percentage change' represent the percentage change of the mean value for the two groups in experiments up to and including No. 17, and the mean percentage change of each plant in relation to its partner in the experiments subsequent to No. 17. The values are not identical but agree closely.

Experiment 18. 7. 10. 32. *Assimilation (7 hours' light)*. Light 1350 f.c.h.  
Temp. 21.0° C.  $N = 9$ .  $n = 9$ . Atmometer 0.686.

	Increase or decrease.	Percentage change.	Probability.
Leaf area . . . .	+ 15.6 cm. <sup>2</sup>	+ 4.84	< 0.01
Dry weight, leaf . .	+ 0.094 grm.	+ 12.89	< 0.01
„ plant . . . .	+ 0.127 grm.	+ 10.79	< 0.01
% water, leaf . . .	- 0.867	- 0.869	< 0.01
„ plant . . . .	- 0.566	- 0.566	< 0.01

Experiment 19 A. 13. 10. 32. *Assimilation (7 hours' light)*. Light 2996 f.c.h. Temp. 21.5° C.  $N = 10$ .  $n = 10$ . Atmometer 0.819.

Leaf area . . . .	+ 11.4 cm. <sup>2</sup>	+ 3.90	0.1
Dry weight, leaf . .	+ 0.099 grm.	+ 20.38	< 0.01
„ plant . . . .	+ 0.139 grm.	+ 19.73	< 0.01
% water, leaf . . .	- 1.482	- 1.337	< 0.01
„ plant . . . .	- 0.897	- 0.897	< 0.01

Experiment 19 B. 13. 10. 32. *Respiration and Translocation (1 hours' darkness)*. Temp. 16.5° C.  $N = 10$ .  $n = 10$ . Atmometer 0.297.

Leaf area . . . .	+ 4.2 cm. <sup>2</sup>	+ 1.54	0.50-0.60
Dry weight, leaf . .	- 0.062 grm.	- 9.45	0.05-0.02
„ plant . . . .	- 0.056 grm.	- 5.11	< 0.01
% water, leaf . . .	+ 1.50	+ 1.50	< 0.01
„ plant . . . .	+ 0.99	+ 0.99	< 0.01

Experiment 20. 24. 10. 32. *Assimilation (7 hours' light)*. Light 1205 f.c.h.  
Temp. 19.5° C.  $N = 6$ .  $n = 6$ . Atmometer 0.386.

Leaf area . . . .	—	—	—
Dry weight, leaf . .	+ 0.039 grm.	+ 7.92	< 0.01
„ plant . . . .	+ 0.051 grm.	+ 9.78	0.05-0.02
% water, leaf . . .	—	—	—
„ plant . . . .	—	—	—



Experiment 21. 2. 11. 32. *Assimilation (7 hours' light)*. Light 1106 f.c.h.  
Temp. 18.5° C.  $N = 6$ .  $n = 6$ . Atmometer 0.350.

	Increase or decrease.	Percentage change.	Probability.
Leaf area . . . .	-19.2 cm. <sup>2</sup>	- 9.03	0.10
Dry weight, leaf . .	+ 0.021 grm.	+ 8.79	0.20-0.10
„ plant . . . .	+ 0.031 grm.	+ 8.36	0.10-0.05
% water, leaf . . .	- 0.25	- 0.25	0.20-0.10
„ plant . . . .	- 0.07	- 0.07	0.60

Experiment 22. 14. 11. 32. *Assimilation (7 hours' light)*. Light 313 f.c.h.  
Temp. 17.0° C.  $N = 15$ .  $n = 15$ . Atmometer 0.296.

Leaf area . . . .	—	—	—
Dry weight, leaf . .	+ 0.002 grm.	+ 0.984	0.6
„ plant . . . .	+ 0.003 grm.	+ 0.890	0.70-0.60
% water, leaf . . .	- 0.055	- 0.055	0.50-0.40
„ plant . . . .	- 0.055	- 0.055	0.40-0.30

Experiment 23. 5. 12. 32. *Assimilation (7 hours' light)*. Light 677 f.c.h.  
Temp. 18.5° C.  $N = 23$ .  $n = 23$ . Atmometer 0.372.

Leaf area . . . .	—	—	—
Dry weight, leaf . .	+ 0.019 grm.	+ 9.37	< 0.01
„ plant . . . .	+ 0.022 grm.	+ 6.71	0.05
% water, leaf . . .	- 0.37	- 0.38	< 0.01
„ plant . . . .	- 0.20	- 0.21	< 0.01

Experiment 24. 19. 1. 33. *Assimilation (7 hours' light)*. Light 398 f.c.h.  
Temp. 17.5° C.  $N = 24$ .  $n = 24$ .

Leaf area . . . .	—	—	—
Dry weight, leaf . .	+ 0.006 grm.	+ 7.49	< 0.01
„ plant . . . .	+ 0.015 grm.	+ 10.91	< 0.01
% water leaf . . .	- 0.56	- 0.56	< 0.01
„ plant . . . .	- 0.38	- 0.38	< 0.01

Experiment 25. 11. 2. 33. *Respiration and Translocation (17 hours' darkness)*. Temp. 11.5° C.  $N = 12$ .  $n = 12$ .

Leaf area . . . .	—	—	—
Dry weight, leaf . .	- 0.007 grm.	- 5.68	0.05-0.02
„ plant . . . .	- 0.010 grm.	- 4.76	0.05-0.02
% water, leaf . . .	+ 0.090	+ 0.085	0.40-0.30
„ plant . . . .	+ 0.08	+ 0.078	0.20-0.10

In the experiments of 17 hours' duration on respiration and translocation the roots are found to gain in dry weight while the leaves loose. This is shown by the following results:

Date of experiment	28.8.32	20.9.32	13.10.32	11.2.33
Temperature °C. .	17.9	10.0	16.5	11.5
<i>Percentage change.</i>				
Whole plant. . . .	- 4.64	- 2.92	- 5.85	- 4.83
Leaf . . . . .	- 10.28	- 7.86	- 10.15	- 5.86
Stem . . . . .	+ 3.10	+ 4.12	- 0.34	- 1.32
Root . . . . .	+ 7.29	+ 6.90	+ 4.80	+ 15.67

## DISCUSSION OF RESULTS.

While the results obtained are to be considered as of a preliminary nature, they indicate that the methods developed are adequate. The more

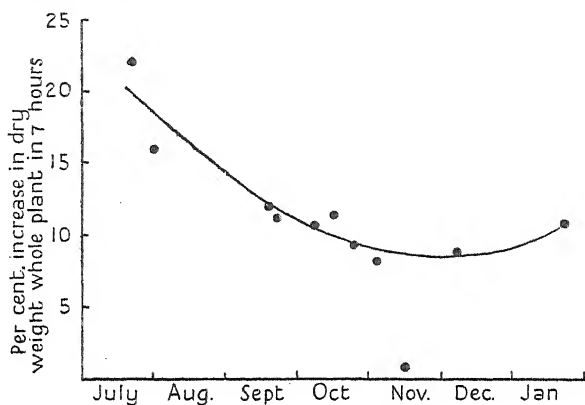


FIG. 8. Seasonal drift of assimilation rate.

important results are shown graphically, the linear graphs being determined from the data by the 'method of least squares'.

*Seasonal drift of assimilation rate.*

This is shown by Fig. 8. Assimilation is plotted without regard to light or temperature conditions. Thus the graph shows the response of the type of plant existing at any particular time of year to the conditions prevailing at that time. It will be observed that the minimum corresponds closely with the 'shortest day', although assimilation rates were in all cases determined over a 7-hour period.

*Assimilation and light.*

Fig. 9 gives some indication of a supra-optimal light, but no satisfactory evidence is yet available as to changes which high light intensities may induce in other factors, such as closure of the stomata, fall in carbon-dioxide concentration in the greenhouse, &c. The similarity of these curves to the curves in Fig. 10 is very striking.

*Fall in water content and light.*

Fig. 10 shows a relation between the fall in water content of the plant tissues at the end of a 7-hour assimilation period and the total light in foot-candle hours during that period. Closure of the stomata or rise of suction pressure of the tissues may in part explain the reduction in fall of water content at high light intensities.

*Fall in water content and assimilation.*

In the assimilation experiments at different light intensities a close positive correlation between the fall in water content of the plant tissues

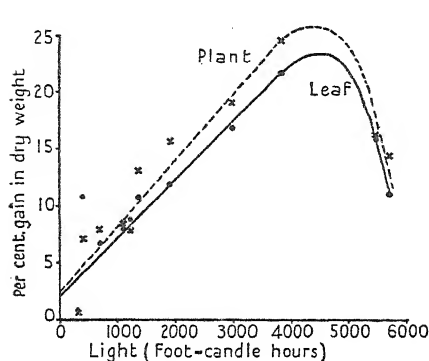


FIG. 9.

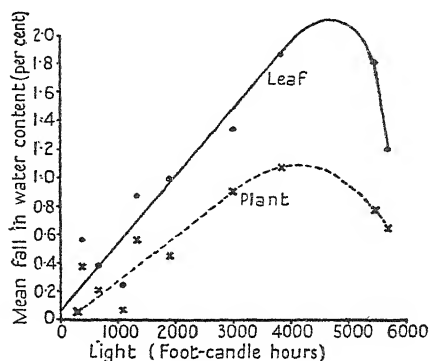


FIG. 10.

FIGS. 9 and 10. Fig. 9. Relationship to light of increase in dry weight of plant and leaf. Fig. 10. Relationship to light of fall of water content of leaf and plant.

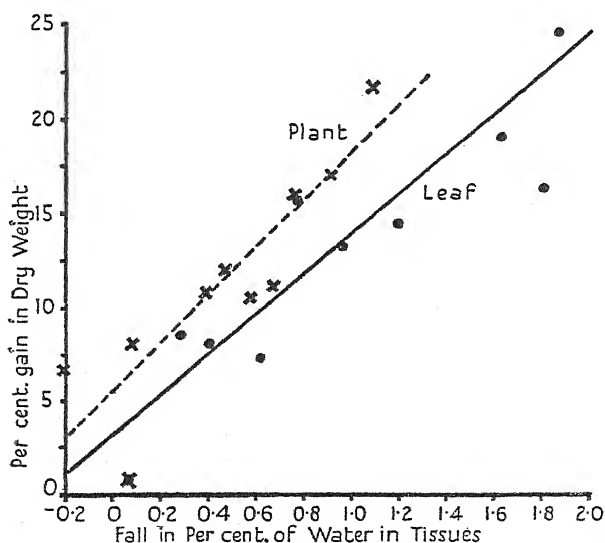


FIG. 11. Relationship between assimilatory rate and fall in water content.

and the gain in dry weight is shown by Fig. 11. This is to be expected from the similarity between the curves for 'Assimilation and Light' and 'Fall in Water Content and Light'.

*Seasonal drift in water content.*

A further correlation between light and water content of the plant tissues may be seen in the seasonal drift in the percentage of water in the

whole plant and in the leaf. In Fig. 12, the percentage of water is plotted against the mean length of day experienced by the plants during their life from germination until the time of sampling. Short day length is

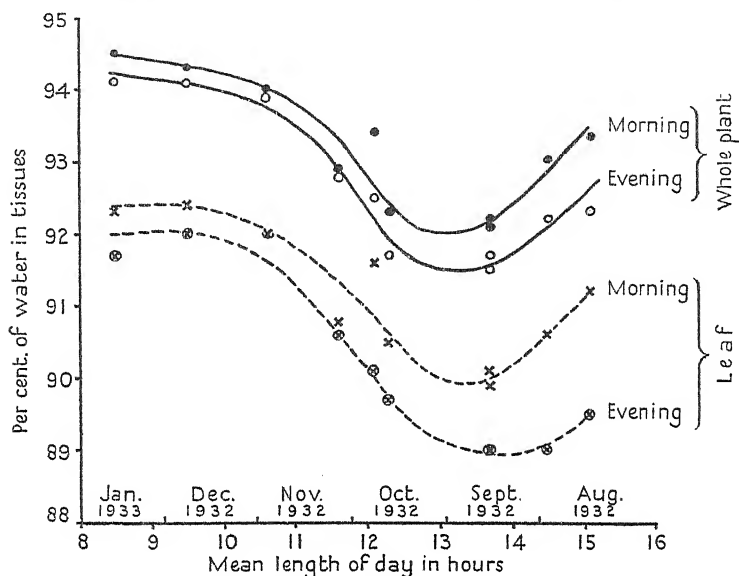


FIG. 12. Seasonal drift of water content of whole plant and of leaf.

associated with low light intensities and a high water content in the plant tissues and results in pale weakly plants of the type described as 'soft' by the growers. In these experiments water reaches a minimum with day lengths of 13 to 14 hours, but with increasing length of day rises again, although the plants concerned are of the sturdy summer type. It will be noticed that the curves for morning and evening water content diverge as the length of day increases. This indicates the drop in water content during the day and is in agreement with the curves shown for assimilation and fall in water content, and for the seasonal drift in assimilation rate. The observations on which these curves are based were made during the autumn and winter months.

#### *Leaf area and light.*

Fig. 13 shows the relation between total light and leaf expansion. It may here be mentioned that there appears to be no correlation between leaf expansion and fall in water content during the day.

#### *Distribution of assimilate during day.*

Fig. 14 shows the distribution of assimilate at the end of the light period. It will be seen that the distribution between stem and leaf is

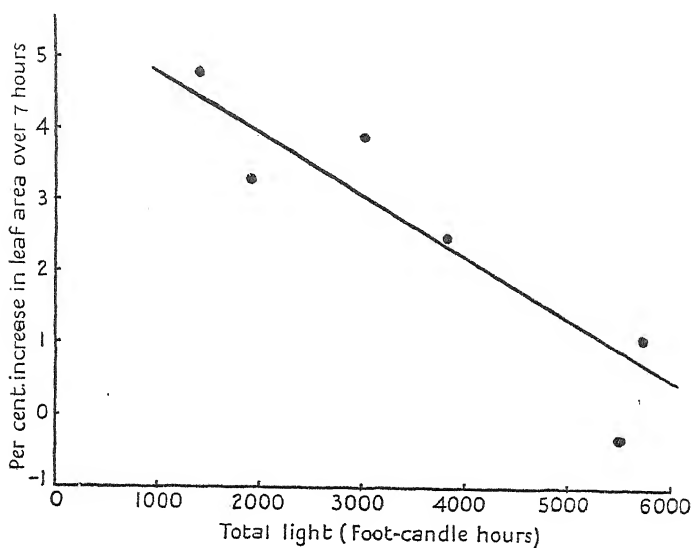


FIG. 13. Graph showing relationship between leaf area increase during a period of 7 hours and the amount of light received.

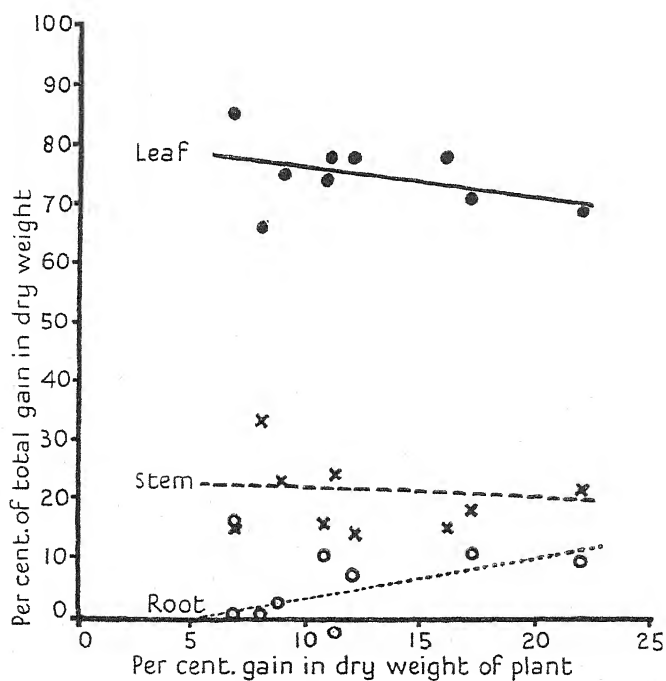


FIG. 14. Distribution of assimilatory material between leaf, stem, and root.

approximately constant. The rise in the proportion taken by the leaf at low assimilation rates may be accounted for by the fall in the proportion taken by the root. As might be expected from the correlation between assimilation rate and light a similar ratio is shown if the distribution of assimilate is plotted against total light. The proportion taken by the root tends to zero when the net plant gain reaches about 5 per cent. It is suggested that at low assimilation rates respiration in the roots may be high enough to mask any increase of weight due to such small amounts of assimilate as may reach the roots.

### *Respiration.*

Perhaps the most interesting feature of the respiration measurements is the magnitude of the losses in a single dark period of 17 hours, the mean value for four determinations being 4.56 per cent. of the total dry weight of the plant.

The authors wish to express their appreciation of the help and advice of Professor V. H. Blackman, under whose direction the work was carried out, and to thank Dr. F. G. Gregory of the Imperial College for advice in relation to a number of problems.

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### SUMMARY.

Methods and technique are described by which assimilation rate, leaf area, water content, respiration, and translocation may be studied in seedling tomato plants under normal cultural conditions under glass.

A new device for the measurement of leaf area is described.

Preliminary determinations of the relation between assimilation, leaf expansion, water content, translocation, respiration, and a number of environmental factors are given.

## NOTE.

**FORMATION OF PYCNIDIA IN CYTOSPORINA LUDIBUNDA BY THE INTERMINGLING OF TWO INFERTILE STRAINS.**—Two saltants of *Cytosporina ludibunda*, CA<sub>2</sub> and CA<sub>4</sub>, separately infertile, have been found to produce pycnidia when their mycelia are allowed to intermingle.

Details of the origin of these strains have been given in a previous paper.<sup>1</sup> Briefly, spores from *Cytosporina ludibunda*, strain C, on plating gave, among others, a strain CA which in its turn produced strain CA<sub>1</sub>, also from spores. The plated spores of CA<sub>1</sub> gave rise to a number of infertile, grey colonies which on subculturing, produced strain CA<sub>2</sub> and also to one minute black colony from which strain CA<sub>4</sub> was eventually obtained.

CA<sub>2</sub> and CA<sub>4</sub> are strikingly different in morphological character. The distinctive features of each are:—CA<sub>2</sub>, *mycelium grey*, zonation wide; CA<sub>4</sub>, *mycelium black*, zonation absent.

The two strains have been grown in a number of synthetic media, but so far in separate culture they have failed to produce pycnidia. On very rare occasions, however, there has appeared on the top of the old inoculum at the centre of a CA<sub>2</sub> culture, a solitary, simple pycnidium which produced 'A' (ovoid) and 'B' (hooked, filiform) spores of the *Phomopsis* type. The spores when plated gave rise to pure, infertile CA<sub>2</sub> only. It should be remembered that 'A' spores germinate freely, and 'B' spores are incapable of germination.

*Meeting of mycelia of CA<sub>2</sub> and CA<sub>4</sub>.* When inocula of CA<sub>2</sub> and CA<sub>4</sub> are placed in the same plate at some distance apart, the developing mycelia advance towards each other, and a number of pycnidia are formed at the line of junction of the two. The pycnidia are usually *large and compound*, and form 'A' and 'B' spores, which are discharged through the ostiole as a yellowish mass. When spores from individual spore masses are plated separately, two types of colony, 'black' and 'grey', corresponding to the two parental types CA<sub>4</sub> and CA<sub>2</sub> appear. No intermediate types occur. The number of platings which have been made, although limited, indicate that usually more CA<sub>2</sub> than CA<sub>4</sub> colonies are produced. The total number of colonies obtained varies according to the size of the spore mass and to the proportion of 'A' and 'B' spores, since the 'B' spores do not germinate.

*Mixed inocula of CA<sub>2</sub> and CA<sub>4</sub>.* Fragments of mycelium of CA<sub>2</sub> and CA<sub>4</sub> when used together as one inoculum, produce a prolifically-sporing culture. Usually a number of sectors are visible, indicating the presence of unequal mixtures of CA<sub>2</sub> and CA<sub>4</sub> mycelium in different regions of the culture. Two kinds of pycnidia are generally produced: (1) *Large, compound pycnidia*, partially embedded in the substratum. These are produced in parts where CA<sub>2</sub> mycelium predominates, and

<sup>1</sup> Das Gupta, S. N., Ann. Bot. xliv. 349-84, 1930.

also along the line of contact of two sectors. They contain both 'A' and 'B' spores, but the 'B' spores are more numerous than the other type. The spores, on plating, give rise to separate colonies of  $CA_4$  and  $CA_2$ , the latter predominating in number. (2) *Minute, simple pycnidia*, superficial. These are produced where  $CA_4$  mycelium predominates. The spores are almost entirely of the 'B' type, therefore only a few  $CA_2$  and  $CA_4$  colonies appear on plating.

There is some experimental evidence that both  $CA_2$  and  $CA_4$  hyphae contribute to the formation of pycnidia, but attempts to determine whether or not hyphae of the two strains fuse together before pycnidia develop proved unsuccessful.

The behaviour of  $CA_2$  and  $CA_4$  resembles that recorded for *Sphaeropsis malorum* Pk. by Mohendra and Mitra.<sup>1</sup> In some respects it is comparable with the heterothallism of various ascomycetes, particularly *Glomerella*,<sup>2</sup> since in this fungus the complementary strains are morphologically distinct. The difference between the two phenomena lies in the fact that while  $CA_2$  and  $CA_4$  unite to form pycnidia, the *Glomerella* strains unite to form perithecia. The stimulus which brings about the formation of pycnidia when the *Cytosporina* mycelia meet is unlikely to be of a sexual nature. It is more probable that this is an example of nutritive heterothallism such as Gwynne-Vaughan<sup>3</sup> suggests is responsible for the formation of perithecia when (+) and (−) strains of an ascomycete meet. Hence the two phenomena may be different aspects of the same problem.

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<sup>1</sup> Mohendra, K. R., and Mitra. M., Ann. Bot. xliv. 541-55, 1930.

<sup>2</sup> Edgerton, C. W., Amer. Journ. Bot. l. 244-54, 1914.

<sup>3</sup> Gwynne-Vaughan, H. C. I., Presidential Address, Section K. Report of the Ninety-sixth Meeting of the British Association, pp. 185-99, 1928.



# A Geographical Survey of the Flora of Temperate South America.

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With two Figures in the Text.

## CONTENTS.

	PAGE
INTRODUCTION . . . . .	691
THE REGION . . . . .	694
Topography . . . . .	694
Geology . . . . .	696
Climate . . . . .	697
Vegetation . . . . .	698
THE FLORA . . . . .	699
General Statistics . . . . .	699
Geographical Analysis of the Genera . . . . .	702
Table of Families and Categories . . . . .	716
Comparison with other Temperate Southern Floras . . . . .	719
SUMMARY AND CONCLUSIONS . . . . .	722

## INTRODUCTION.

THE most conspicuous feature of world geography is the almost continuous circle of land in the higher latitudes of the northern hemisphere, and the almost complete circle of ocean in the corresponding latitudes of the southern hemisphere. From the botanical point of view this has the effect of giving, in the southern half of the world, three very marked temperate floras widely separated from one another, the S. American, the S. African, and the Australasian, the latter comprising southern Australia and New Zealand. The richness and floristic peculiarities of these three floras has made them of special interest to the plant-geographer, and they have been much studied. All three, however, have not received the same amount of attention. South Africa and Australasia are parts of the British Empire, and their floras have been particularly investigated by British

botanists, and they are much better known, at any rate to Europeans, than the flora of S. America. The temperate part of S. America is, moreover, very heterogeneous, being made up of five mutually independent areas, Paraguay, Uruguay, Argentine, Chile, and the Falkland Islands.

Presumably largely because of this heterogeneity there is no general flora available of temperate S. America as there is for S. Africa and for Australasia, and there is at present no apparent prospect of such a flora. There are, it is true, floras of all but one of the constituent areas, namely Paraguay, and even in this case much collected material, but two of these floras, those of Uruguay and of Chile, are incomplete and not, at present, in course of completion.

As a result of this the general constitution and floristic features of the flora of temperate S. America are not nearly so well known as are those of S. Africa and Australasia, and a detailed comparison of all three is neither easy nor satisfactory. This is particularly unfortunate because of the great theoretical interest of the temperate S. American flora, especially in relation to the problems of the origin and relationships of the southern floras and their phylogenetic connexions with those of the north.

It was therefore felt that a statistical summary of the existing knowledge of the flora of temperate S. America might provide additional material for such a comparison of the southern floras, and might throw considerable light on some of the problems mentioned. With this end in view the survey here described was undertaken.

To attempt anything in the nature of a systematic revision of the flora of so large a region as that part of S. America outside the tropics, including, as it does, many thousands of species, was clearly impossible, and it was necessary to compile the desired statistics by other means. The method adopted finally was as follows.

First, as complete as possible a list of floras, plant-lists, and descriptive notes relating to the region was made, and from these sources a list of the genera occurring therein was compiled. This list was then augmented from such publications as the *Pflanzenfamilien* and the *Pflanzenreich* Monographs, the *Vegetation der Erde* and the *Vegetationsbilder*. This list was then examined, and with the help of various authorities the synonyms were removed, and the genera represented only by introduced species or by species of doubtful status were eliminated. There then remained a list of indigenous genera as complete as it could reasonably be made short of a revision of the whole flora, and this list became the basis for further treatment.

Next, the distribution of all the species throughout the world of all the genera in the list was compiled from the original volumes and first seven supplements of the *Index Kewensis*. For this purpose the world was considered as divided into sixteen regions:

Temperate South America.	Tropical East Asia.
Tropical South America.	Tropical Africa.
Central America.	South Africa.
West Indies.	Madagascar &c.
North America.	Australia.
Europe and Western Asia.	New Zealand.
Central Asia.	Polynesia.
Temperate Eastern Asia.	

Lastly, the distribution of as many genera as possible was verified and corrected from monographs, floras, examination of herbarium specimens, and from collected notes.

The final result was a list of genera with the world distribution of all the species contained therein, and from this list the figures and statements in the following pages were taken.

It is important to realize at once the limitations of this method of work. In the first place, although it gives considerable information as to the absolute and proportional distribution of the genera it clearly does not provide sufficiently detailed data for any significant analysis of species, and for this reason consideration here is practically confined to genera. Reference to species is made only when the distribution of a genus itself is insufficient to illustrate points of particular interest. In the second place the Index Kewensis is simply a list of the species which have been described in the various genera, together with a rough indication of their locality. It makes no attempt to express opinion on the value of species, although it gives new combinations. Furthermore, since the species are recorded, except in the case of the original volumes, only when newly described, subsequent increases in range are not evident. In the case of comparatively wide areas, such as are here under consideration, this is probably not of very serious importance. The Index also, of course, makes no discrimination between authors of species or between the various conceptions of the term 'species'. This is perhaps the most serious limitation in the method, but it is one which can be overcome only by a systematic revision of the groups concerned, and this is manifestly impossible. As a matter of fact, it is surprising to find how frequently the data of a given genus taken from the Index compares closely with the data for that genus taken from an independent monograph. It may also be noted that since all the described species of a genus are considered, the numbers so derived are likely to be above the actual number, since more careful study would probably lead to the discovery of a certain amount of synonymy. On the other hand, subsequent study will almost certainly reveal hitherto undescribed species which will tend to balance the loss by synonymy.

Another minor limitation of the method is that the species distribution

is generally given in the Index in very concise form, for obvious reasons of economy. Fortunately the political divisions of S. America are, for the most part, so closely correlated with the tropic of Capricorn that no great difficulty arises except in the two regions Chile and Brazil. Both these countries have actually both tropical and temperate parts. This difficulty has been met by a slight modification of the strictly geographical conception of temperate S. America. Chile, which extends some distance into the tropics, is treated as entirely temperate, and Brazil, which has three provinces south of the tropic is treated as entirely tropical and outside the scope of this survey. This modification has, in fact, been rather beneficial than otherwise, because it gives a more natural floristic area than would have resulted from a strict adherence to latitude. That part of Chile within the tropic is on account of its altitude predominantly temperate. That part of Brazil outside the tropic is clearly but an outlying part of the general Brazilian floristic region, and to have included it would have been to add to the list many genera which have no claim to be considered as real constituents to the flora of temperate South America.

The following analysis is mainly a matter of figures, and there is no need to stress the fact that figures may mean much or little. It must, however, be borne in mind that the figures used are not employed or are not intended to be employed in any absolute sense, except in the comparatively few cases where they are so small or so definite as to be free from misinterpretation. As a rule, they are used simply as a means of comparing and contrasting certain groups or aspects of the flora as a whole. It is these similarities and differences which form the most important part of the analysis, and for this purpose the figures are adequate.

It has also been thought best to quote figures exactly as they emerge from the analysis rather than to convert them into round numbers. The exactness of many of the figures must therefore be viewed in this light, and not as any indication of extreme and invariable accuracy.

It must further be remembered that for the purposes of the statistics it has frequently been necessary to take up a definite, if somewhat arbitrary, attitude upon more or less undecided questions. This has been done with due regard for the available evidence, but it is inevitable that many details will be liable to criticism. It is, however, believed that these will be altogether insignificant compared with the general analysis.

## THE REGION.

### *Topography.*

The general outline of temperate S. America is that of an irregular triangle with one side, that running south-east to north-west, considerably shorter than the other two. These latter are nearly equal, and one runs north and south. The whole area is divided into two very unequal parts

by the enormous chain of the Andes, which lies north and south close to and parallel with the west coast. This mountain system is widest in the north, and narrows down almost to a point at Cape Horn. It is also less

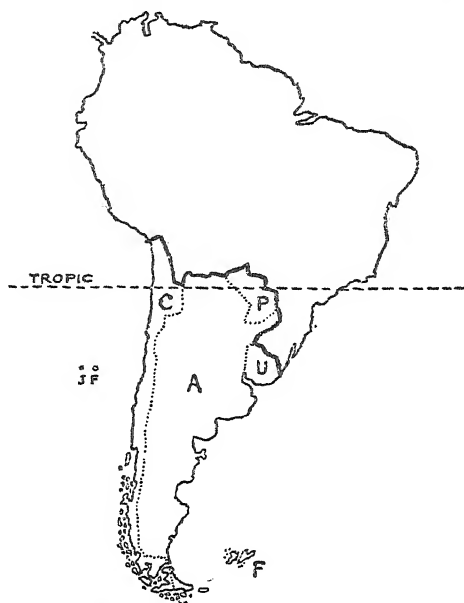


FIG. 1. Map showing temperate South America as defined in this paper. The letters indicate the political boundaries of the region.

elevated towards the south. The narrow strip of land to the west of these Andine Cordilleras is not more than one hundred miles wide, and is somewhat elevated. In the south it is clearly the remains of a valley between two mountain systems, the western of which is now represented by the fringing islands of the Chilian coast.

The much larger area to the east of the mountains is easily divisible into three. First, there is the hilly country east of the R. Uruguay, marking the southern limits of the Brazilian highlands. Second, there are the flat lowlands of the rivers Parana, Paraguay, Uruguay, Colorado, and Negro extending to latitude 40 S. The only conspicuous feature in this area is an outlier of the Andes in the northern Argentine. Third and south of 40 is an elevated foot-hill plain broken up by valleys.

Below about 40 S. the west coast is fringed with islands, of which the largest is Chiloe. In latitude 34 and four or five hundred miles out into the Pacific lie the small but very interesting island group of Juan Fernandez. On the east coast there are practically no islands except the Falklands, which form a broken continuation of the recurved tip of Patagonia, a topographical feature of great interest in relation to theories of continental displacement.

*Geology.*

It is difficult to give any concise but coherent account of the geology of so large an area as temperate S. America, especially as our knowledge of it is by no means complete. If, however, S. America is for the moment, considered as a whole the main features of its geological construction can be indicated fairly shortly. It may be divided into three regions.

The first is the western chain of mountains, and the geology of these is so intricate that only the most salient features can be mentioned. These are briefly that the foundations of the area are extremely old rocks forming a very ancient land surface which has undergone successive elevations from time to time. The last and, as far as we can tell now, by far the most important mountain building was that of the Tertiary, which commenced in the Miocene, and which continued until recent times. To this, aided as it has been by land elevation and volcanic activity, is due the present mountain system. That the elevation of at least many of the mountains post-dates the Mesozoic is shown by the presence of Jurassic and Cretaceous marine deposits at great heights. The south-western seaboard, which is much less elevated, represents in particular the remains of a very ancient and long exposed land surface.

The second area is that of the north-eastern or Brazilian highlands. This again is an extremely old land surface, and much of it has been unsubmerged since the time before the Palaeozoic period. Other parts of it are at present covered with Palaeozoic and Mesozoic deposits.

The third region is a long lowland stretch separating the two already described. It extends from south Argentine to Trinidad, follows the outline of the west coast, and becomes gradually narrower towards the north. Geologically this is much younger than either of the other regions. Much of its central part is covered with rocks of Tertiary age, but in the north and south they are even younger, being for the most part diluvial and alluvial.

These paragraphs can be condensed into the statement that the geological structure of S. America is that of a vast geosyncline or trough with its longer axis running roughly north and south in a line parallel with the west coast. The edges of this syncline are the very ancient land surfaces of the Brazilian highlands on the east and the coastal cordilleran belt on the west. The whole continent has undergone great variations in level with the result that the trough itself has been constantly submerged. Its latest elevation appears to have been comparatively recent, and most of it is covered with fresh water deposits.

As regards the region actually under discussion in this paper, the Uruguayan region represents the only part of the eastern syncline in it and the cordilleras of the west represent the western edge. The area between forms part of the trough.

With regard to the history of the S. American vegetation and of Angiosperms in general three points of great importance emerge from this topographical and geological sketch. First and foremost it may be accepted that some kind of land surface existed in both eastern and western S. America throughout the history of the flowering-plants, and probably for much longer. This is particularly true of parts of Chile and Uruguay. Secondly, although there has been a mountain range system on the west coast during most of their history the development of the enormous massif of the Andes dates only from the latter half of the Tertiary. Thirdly, the major part of the area between those already mentioned has been successively raised and lowered, and is presumably by far the youngest part of the whole, perhaps dating as an existing land surface since the Pliocene. It may be added, in conclusion, that there is every reason to believe that the older parts of the continent were, during the Tertiary, considerably larger than they are now, and included many of the islands now lying off the coasts.

#### *Climatic.*

Owing to its great latitudinal extent and to the high mountain wall on the west, temperate S. America has a very varied range of climatic conditions.

The mean annual temperature varies from above 75° F. in north-western Argentine to about 40° F. in the extreme south. The annual maximum varies from 104° F., again in the north-western Argentine, to 70° F. in the south. There is also a great difference in the annual range of temperature. This is greatest (81° F.) in the central part of the eastern Argentine, and this decreases both north and south to 45° F. at the extreme distances. The annual minimum ranges from 32° F. to 23° F. so that throughout the whole region the temperature is liable, at some period of the year, to drop to freezing-point.

The total annual rainfall is greatest in the southern half of the western coast where it exceeds 80 in. From this line the figure falls rapidly to a minimum of under 10 in., a figure which prevails over a wide zone from southern Argentine to northern Chile. North of latitude 34 a similar figure occurs in the Chilean coast zone, and this area is one of the driest and least-fertile in the whole world. It is here, in the Atacama desert, that the great nitrate deposits are found. East of this dry zone the rainfall rises again, but less rapidly, to a maximum of between 40 and 60 in. in eastern Uruguay. From these data it will be seen that the rainfall follows a series of parallel zones running almost north and south with the largest and driest of them in the centre and covering most of Argentine and northern Chile.

Even more important than the absolute rainfall, is the seasonal

distribution of the precipitation, and here again different parts of the area vary greatly. In the west, south of 37 S., and in the east also, south of 50 S., there is rain at all seasons with a maximum in winter and autumn. On the west between 30 and 37 S. the rain falls mostly in winter, and the climate is of the 'Mediterranean' type. Winter rain also falls in certain small areas of the eastern coast. Over the remainder of the region, including the very dry parts, the rainfall, such as it is, is periodic and reaches a maximum in summer, with dry winters and springs. There are thus three types of precipitation. The widest spread, which covers most of the east, is summer rain. A portion of the west has winter rain, and the remainder of the west and the extreme south has rain at all seasons.

The proportion of cloudiness varies a good deal. South of 47 S. it is at its highest, corresponding to the figures for the north of Scotland. In central Argentine it reaches its opposite extreme in a value of 3 as compared with 7. Further north from here cloudiness again increases.

In the southern part of the region there are persistent westerly winds, and these are particularly marked and important in the Falkland Islands.

### *Vegetation.*

Botanically, temperate S. America can be divided into three main areas, an alpine area forming a very long and narrow triangle gradually thinning to a point at about latitude 45 S.; a region to the east of this mountain backbone; and a region to the west of it. The latter two can be further subdivided, the one into four and the other into three.

In the west there is first the desert vegetation which extends from northern Chile as far as 30 S. Then between 30 and 37 S. the vegetation is sclerophyllous and of the characteristic kind known as 'Mediterranean', correlated with the occurrence of winter rainfall. South of this again from 37 S. to Cape Horn the vegetation is temperate forest.

On the east of the Andes the different vegetational regions vary much in size. In Paraguay and north-eastern Argentine is a considerable area of warm temperate forest, containing also wide areas of swamp. South of this, and comprising most of Uruguay as well as that part of Argentine immediately to the west and south, is the *pampa*—a great plain of grass-land which economically, is one of the most important parts of the world. Then in the south of Patagonia is a narrow region, vegetationally best called moorland. This is found also on the west of the continent beyond the southern limits of the separating alpine zone. The remainder, and by far the larger part of the east, comprising nearly all Argentine, is semi-desert. This varies much locally, and particularly in parts of western Argentine where there are considerable stretches of salt pans.



## THE FLORA.

At the end of this portion of the paper a table is given (p. 716) containing all the families of the flora and the numbers of the genera by which they are represented. The genera are also tabulated according to a number of geographical categories which will be described later. The table shows, in concise form, many of the points described in the following pages and should be studied in conjunction with a perusal of them.

*General Statistics.*

According to the list of genera compiled by the method described in the introduction, the flora of temperate S. America as there defined contains 1478 genera. These are contained in 177 families, giving an average of 8.35 genera per family. The number of species is somewhere between 12,000 and 12,500. Taking the mean between these two numbers, each genus has, on the average, 8.3 species. This figure is rather low when compared with the corresponding value for the whole world, which is usually considered to be about 12.

Of the families, 32 are Monocotyledons and 145 are Dicotyledons. By far the largest of the former is the Gramineae with 106 genera. Then comes the Orchidaceae with 41 genera, followed by the Liliaceae with 26, and the Amaryllidaceae, Bromeliaceae, and Cyperaceae with about 20 each. 10 families are represented by one genus each only.

By far the largest family of the Dicotyledons is the Compositae with 180 genera, followed at a considerable interval by the Leguminosae with 93. Next come the Rubiaceae and Solanaceae with 39 each, and after them the Asclepiadaceae, Boraginaceae, Bignoniaceae, Caryophyllaceae, Cruciferae, Euphorbiaceae, Labiatae, Malpighiaceae, Scrophulariaceae, and Umbelliferae with between 20 and 30 each. 39 families of Dicotyledons are represented by one genus each only.

Eleven families in the list, namely Bromeliaceae, Cactaceae (except for the doubtful genus *Rhipsalis*), Myzodendraceae, Malesherbiaceae, Calyceraceae, Nolanaceae, Lacistemaceae, Vochysiaceae, Martyniaceae, Caricaceae, Aextoxicaceae, and Tropaeolaceae, are confined to America. Of these the first two have by far the largest representation. Two only, Myzodendraceae with two genera and Aextoxicaceae with one, are confined to temperate S. America. In terms of families the endemic element in the flora is therefore very small.

The Compositae, the Gramineae and the Leguminosae are outstandingly the largest families in genus numbers. They are so also in number of species, the Compositae having over 2,000, the Gramineae over 1,000, and the Leguminosae over 800. Next come the three families Solanaceae, Rubiaceae, and Orchidaceae. The two latter are widespread and large

families, and their representation is not remarkable, but the first has in the whole world not much above 80 genera, so that it has a 50 per cent. representation in the flora and is one of its major features. The Cyperaceae, considering the small number of its genera, is especially well represented. The Glumiflorae as a whole form a second major feature of the flora.

No very widespread families are entirely absent from the flora, but among large families which are particularly poorly represented the Araliaceae, Crassulaceae, Menispermaceae, Papaveraceae, and 'Amentiferae' may be mentioned.

The constituent political parts of the region vary a good deal in the number of genera comprising their floras. The method of compiling the data used here does not permit of any very accurate statement being made on this point, but the position appears to be somewhat as follows :

Argentina	has about	925	genera
Paraguay	„ „	750	„
Chile	„ „	660	„
Uruguay	„ „	300	„
Juan Fernandez		74	„
Falklands	„ „	93	„

From these figures it is seen that the richness of the flora, in genera at least, decreases from north to south. Paraguay has, considering its size, much the largest number. Patagonia, on the other hand, about twice the size, has less than 500, and the Magellan region south of 52 S. has under 200.

Of the whole 1478 genera in the list a significant proportion, between  $1/6$  and  $1/5$ , are represented only by non-endemic species.

Of all the genera only 52 are represented in temperate S. America by more than 50 species, or at least by more than 50 endemic species, and of these 25 have less than 70 species. If the number is raised to 100 only 14 genera exceed it. This number must, considering the number of genera in the whole flora and the wide extent of the region, be looked upon as very small, and accounts to a great extent for the rather low generic species average.

A list of these 52 genera together with their world distribution is given below, and from it it will be seen that the genus *Senecio* stands out head and shoulders above the rest and without any notable rival. Of this genus 446 species have been described from temperate S. America, and this is approximately 17 per cent. of all the species in the genus. Next to it, but far behind, is *Adesmia*, a genus of the Leguminosae belonging to the group of the *Hedysareae*. This is one of the most characteristic and conspicuous genera in the flora because it is the nearest approach to a really large endemic genus such as occurs in the other southern temperate floras of S. Africa and Australia. Actually it is western and montane and ranges up the

Andes into tropical latitudes, but apart from this it is an endemic genus. The third largest genus, *Oxalis*, is also of interest because of the companion centre of dense species population which it has in S. Africa, but it is far less restricted (it is nearly cosmopolitan) and has, in America, even more species within the tropic than outside. This last remark applies also to *Solanum*. *Acacna* and *Calceolaria*, on the other hand, are definitely southern genera. The very wide distribution of many of the genera in this list should be noted.

*Genera represented in the Flora of Temperate S. America by 50 or more Species, together with their Families and World Distribution.*

<i>Senecio</i>	446 spp.	Compositae	cosmop.
<i>Adesmia</i>	260	Leguminosae	South America
<i>Oxalis</i>	205	Oxalidaceae	cosmop.
<i>Solanum</i>	165	Solanaceae	cosmop.
<i>Calceolaria</i>	141	Scrophulariaceae	America-New Zealand
<i>Acacna</i>	127	Rosaceae	southern temperate
<i>Baccharis</i>	125	Compositae	America
<i>Astragalus</i>	123	Leguminosae	pan-temperate
<i>Eugenia</i>	120	Myrtaceae	pan-tropical
<i>Chloraca</i>	120	Orchidaceae	America
<i>Verbena</i>	110	Verbenaceae	America and N. Temperate
<i>Plantago</i>	108	Plantaginaceae	pan-temperate
<i>Haplopappus</i>	107	Compositae	America
<i>Echinocactus</i>	103	Cactaceae	America
<i>Calandrinia</i>	96	Portulacaceae	America-Australia
<i>Poa</i>	94	Gramineae	cosmop.
<i>Viola</i>	91	Violaceae	pan-temperate
<i>Leuceria</i>	88	Compositae	South America
<i>Dioscorea</i>	86	Dioscoreaceae	pan-tropical
<i>Valeriana</i>	87	Valerianaceae	temperate
<i>Agrostis</i>	85	Gramineae	cosmop.
<i>Opuntia</i>	85	Cactaceae	America
<i>Stipa</i>	84	Gramineae	pan-tropical
<i>Gnaphalium</i>	78	Compositae	cosmop.
<i>Erigeron</i>	74	Compositae	cosmop.
<i>Loasa</i>	71	Loasaceae	tropical America
<i>Cereus</i>	71	Cactaceae	America
<i>Sisyrinchium</i>	69	Iridaceae	America, one Europe
<i>Ranunculus</i>	65	Ranunculaceae	cosmop.
<i>Polygala</i>	62	Polygalaceae	cosmop.
<i>Nassauvia</i>	62	Compositae	endemic
<i>Mutisia</i>	61	Compositae	South America
<i>Paspalum</i>	61	Gramineae	pan-tropical
<i>Festuca</i>	61	Gramineae	cosmop.
<i>Conyza</i>	61	Compositae	pan-tropical
<i>Cardamine</i>	61	Cruciferae	cosmop.
<i>Carex</i>	60	Cyperaceae	cosmop.
<i>Sisymbrium</i>	60	Cruciferae	pan-temperate
<i>Lycium</i>	57	Solanaceae	pan-temperate
<i>Oxyptalum</i>	56	Asclepiadaceae	South America
<i>Berberis</i>	54	Berberidaceae	South America, N. Temperate
<i>Eupatorium</i>	54	Compositae	America and N. Temperate
<i>Ipomoea</i>	54	Convolvulaceae	pan-tropical
<i>Panicum</i>	54	Gramineae	pan-tropical
<i>Astroemeria</i>	52	Liliaceae	South America
<i>Hippeastrum</i>	52	Liliaceae	America

<i>Myrtus</i>	52	Myrtaceae	America, Asia, Australia
<i>Azorella</i>	51	Umbelliferae	S. America-New Zealand
<i>Cristaria</i>	51	Malvaceae	South America
<i>Lippia</i>	51	Verbenaceae	pan-tropical
<i>Vicia</i>	51	Leguminosae	America, N. Temperate
<i>Escallonia</i>	50	Berberidaceae	South America

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The distribution outside temperate S. America of the 1478 genera in the list can be summarized by a table showing the number of these genera which occur in the other major divisions of the world. Using the regions which have already been listed on p. 693 the figures are as follows:

About 1,100 occur also in tropical South America.

"	550	"	West Indies.
"	560	"	North America.
"	875	"	Central America.
"	260	"	Mediterranean and West Asia.
"	240	"	Europe.
"	300	"	temperate East Asia.
"	415	"	tropical East Asia.
"	250	"	Central Asia.
"	400	"	tropical Africa.
"	325	"	South Africa.
"	270	"	Madagascar, &c.
"	340	"	Australia.
"	165	"	New Zealand.
"	230	"	Polynesia.

The genera may also be considered in relation to the regions in which the greatest numbers of endemic species occur. The figures are:

About 450 genera have most endemic species in tropical S. America.

"	100 in temperate South America.
"	13 in the West Indies.
"	60 in Central America.
"	90 in North America.
"	20 in Europe.
"	33 in Mediterranean and West Asia.
"	8 in Central Asia.
"	10 in temperate East Asia.
"	80 in tropical East Asia.
"	70 in tropical Africa.
"	10 in South Africa.
"	1 in Madagascar, &c.
"	15 in Australia.
"	10 in New Zealand.
"	2 in Polynesia.

The remainder have no marked centre of population.

### *Geographical Analysis of the Genera.*

In order to investigate the relations of the flora further it is necessary to arrange the genera into groups or categories according to their world distribution so that the relative prevalence of the various types becomes

apparent. They are therefore here divided into the following nine categories.

Category 1. Genera endemic to temperate S. America.

Category 2. Genera confined to S. America.

Category 3. Genera occurring in S. America and in one or both of Central America and the West Indies.

Category 4. Genera ranging through America from south to north.

Category 5. Genera found more or less throughout the tropics of both hemispheres.

Category 6. Genera incompletely tropical in distribution.

Category 7. Genera either pan-temperate or more or less cosmopolitan.

Category 8. Genera incompletely temperate in distribution.

Category 9. Genera entirely or almost entirely confined to the southern hemisphere.

These categories naturally vary considerably in size, and most of them are further subdivided. The comparative numbers of each and their constitution can be seen in the final table as well as in the following pages.

*Category 1. Genera endemic to temperate S. America.*

The number of genera in this category is 284, of which about three-fifths are monotypic.

Their distribution over the region is roughly as follows:—Chile has 125 genera, Argentine has 65, Paraguay has 1, Juan Fernandez has about a dozen and Uruguay three or four. The remainder are not confined to any one political area.

These figures show clearly that as far as genera are concerned the strongest endemic element is in Chile, where it is about 20 per cent., Juan Fernandez coming next with a figure of about 16 per cent. Argentine with 7.5 per cent., and Paraguay with 2.5 per cent. come far behind, and Uruguay with only some 1 per cent. is weakest of all. These values are obviously related to the topographical or geographical isolation of the different areas. The conditions in Chile distinguish it sharply from the rest, and the islands of Juan Fernandez are very isolated.

The 284 endemic genera account for about 900 species, giving an average of only a little more than 3. The largest genus is *Nassauvia* (Compositae) with 62 species. Next come *Cajophora* (Loasaceae) and *Mulinum* (Umbelliferae) with 35 and 31 respectively. *Azara* (Flacourtiaceae), *Asarca* (Orchidaceae), *Piptochaetium* (Gramineae), and *Triptilium* (Compositae) have between 20 and 30 species each. *Anarthrophyllum* (Leguminosae), *Benthamiella* (Solanaceae), *Closia* (Compositae), *Cruickshankia* (Rubiaceae), *Cochranea* (Boraginaceae) *Dendroseris* (Compositae),

*Hexaptera* (Cruciferae), *Menonvillea* (Cruciferae), *Myzodendron* (Myzodendraceae), and *Nardophyllum* (Compositae) have between 10 and 20 species. None of these is confined to any one political area.

No less than 56 of the genera belong to the Compositae, a notably large proportion. Liliaceae has 16, Solanaceae has 14, Gramineae has 13 and Leguminosae has 10. The high figure for the Solanaceae is striking. 77 families are represented in the category, but of these 36 have only one genus. Large families quite unrepresented are the Cyperaceae, Urticaceae, Araliaceae, Ericaceae, Gentianaceae, Convolvulaceae, and Acanthaceae.

If the floristic distribution of the genera is considered the following figures result. 60 are Monocotyledons, 112 are Archichlamydeae, and 115 are Sympetalae, the size of the last figure being chiefly due to the Compositae. As regards the orders of Angiosperms 7 out of 11 in the Monocots, 17 out of 30 in the Archichlamydeae, and 8 out of 10 in the Sympetalae are represented. The Campanulatae, Tubiflorae, and Liliiflorae are specially conspicuous in the order named. Other important orders are the Glumiflorae, Centrospermae, Rosales, and Geraniales. As regards families the representation is 15 out of 45 in the Monocots, 46-189 in the Archichlamydeae, and 16-52 in the Sympetalae.

#### *Category 2. Genera confined to S. America.*

The number of genera in this category is 233.

These can be again divided according to their distribution into those which are predominantly tropical, numbering 99; those which are predominantly temperate, 35; and those which are neither 99.

The data of the genera in this category are taken mainly from their endemic species, and it may be that in certain genera the occasional extension of a species to Central America or the West Indies has been disregarded.

The category does not include many large genera, the only ones represented in the temperate S. American flora by more than 50 species being *Leuceria* with 88 temperate and a few tropical species; *Mutisia* with 65 temperate and 31 tropical; *Oxyptalum* with 56 temperate and nearly 100 tropical, and *Alstroemeria* with about 50 of each and many of the temperate in Uruguay. The two first-named belong to the *Mutisieae* section of the Compositae, a section which is specially characteristic of S. America. *Escallonia* and *Cristaria* also have about 50 temperate species.

The distribution of the genera in the three subdivisions mentioned above is of considerable interest. Those which are predominantly southern are nearly all western (Chilian), while those which are predominantly northern are nearly all eastern (Argentine &c.). Those which are neither are for the most part eastern. This seems to provide a definite piece of evidence

that the direction of plant spread has been northwards on the west and southwards on the east.

Many of the genera in this category are strongly developed in the tropics and are represented in the temperate by a few species only. Such are *Agarista* (Ericaceae) with some 20 species, none endemic below the tropic: *Qualea* (Vochysiaceae) with 54 tropical species; *Caladium* (Araceae) 34 tropical species and *Kielmeyera* (Guttiferæ) 23 tropical species. On the other hand, most of the genera well represented in the tropics of S. America are found also in Central America or in the West Indies and therefore appear in the next category.

Several genera such as *Adesmia* come into this category because they have Andine species at high altitudes and high latitudes. They do not possess really tropical species.

79 families are represented among the 233 genera. Once more the Compositæ comes first with 28 genera, but with a smaller preponderance than before, being followed by Leguminosæ with 15 and Asclepiadaceæ with 14. The high places of the two last named clearly reflect the tropical relation of the category. 41 families show one genus only.

*Category 3. Genera occurring in S. America and in one or both of Central America and the West Indies.*

The number of genera in this category is 229.

They may be divided into four classes:

1. Genera of temperate South, tropical South, Central America, and West Indies. About 115.
2. Genera of temperate South, tropical South, and Central America. About 80.
3. Genera of temperate South, tropical South, and the West Indies. About 25.
4. Genera of temperate South and Central America. About 10.

This category is in some ways the most unsatisfactory as regards details because it has not been possible to determine the ranges of many of the non-endemic species fully. The figures given are those of endemic species or as given in the Kew Index. It is quite possible and, indeed, almost inevitable that some of these species reach other areas. This must be remembered when the data given are being considered. Despite it the figures are sufficient to show many interesting points.

It will be noticed how many genera are absent, at least as regards endemic species, from the West Indies. This is perhaps the major feature of the category and seems to indicate the presence of two elements, one of western tropical America tending to be absent from the West Indies, and

one of eastern tropical America often absent from Central America. The latter is much the smaller. It also indicates that the West Indies have not lain in the direct path of plant movement north and south.

Subdivision 4 should be treated with special caution, and it is probable that most of the genera therein should be placed in subdivision 2, that is to say, that they have species in tropical S. America although this does not appear from the statistics of their endemic species. In all but one of these genera endemic species have been described from temperate S. America.

The great majority of the genera in this category are centred in the tropics and have but a meagre representation in the temperate region. Such are *Gesneria* (Gesneriaceae), *Leandra* and *Miconia* (Melastomaceae), *Palicourea* (Rubiaceae), *Pleurothallis* (Orchidaceae), *Siphocampylus* (Campanulaceae), and *Vochysia* (Vochysiaceae). The opposite condition of predominance in temperate latitudes is shown notably by two genera only, *Echinopsis* (Cactaceae) and *Proustia* (Compositae).

In this category the Orchidaceae take pride of place with 21 genera—4 more than the Compositae and Rubiaceae. Leguminosae and Bignoniaceae are also well marked.

In all 72 families are represented, 35 of them by one genus only.

*Category 4. Genera ranging through America from south to north.*

The number of genera in this category is 184.

The category can be further subdivided according to the distribution of the genera in America as follows :

1. Genera in all five regions of America i.e. in temperate South, tropical South, Central, West Indies, and North. 83 genera.
2. Genera in temperate South, tropical South, Central, and North. 67 genera.
3. Genera in temperate South, tropical South, and North. 11 genera.
4. Genera in temperate South, Central, and North. 7 genera.
5. Genera in temperate South and North. 16 genera.

There are no genera having the ranges :

Temperate South, Central, West Indies, and North.

Temperate South, tropical South, West Indies, and North.

As in category 3 the data are compiled from the endemic species and the same reservations apply here also.

A major feature here, as in category 3, is the comparatively small representation of the West Indies. It will be seen that only 83 out of 184 genera reach that region, and all these occur also in all the other divisions of the continent.



The genera of subdivision 3 are:

Compositae—*Chaetanthera*, *Soliva*.  
 Boraginaceae—*Amsinckia*.  
 Hydrocharitaceae—*Elodea*.  
 Ericaceae—*Gaylussacia*.  
 Iridaceae—*Herbertia*.  
 Malvaceae—*Modiola*.  
 Pontederiaceae—*Pontederia*.  
 Umbelliferae—*Bowlesia*.  
 Gramineae—*Zizaniopsis*, *Anthochloa*.

The genera of subdivision 4 are:

Gramineae—*Scleropogon*.  
 Papaveraceae—*Argemone*.  
 Polygonaceae—*Eriogonum*.  
 Onagraceae—*Gaura*.  
 Leguminosae—*Hosackia*.  
 Cruciferae—*Lesquerella*.  
 Chenopodiaceae—*Spirostachys*.

The genera of subdivision 5 are:

Compositae—*Blennosperma*, *Lasthenia*, *Madia*, *Psilocarphus*, *Troximon*.  
 Gramineae—*Munroa*, *Panicularia*.  
 Onagraceae—*Gayophytum*, *Boisduvalia*.  
 Polygonaceae—*Chorizanthe*.  
 Campanulaceae—*Downingia*.  
 Saxifragaceae—*Lepuropetalon*.  
 Chenopodiaceae—*Nitrophila*.  
 Valerianaceae—*Plectritis*.  
 Polemoniaceae—*Collomia*.  
 Boraginaceae—*Allocaryastrum*.

These three subdivisions between them include all the genera which show discontinuous distribution between temperate South and North America. They number 49, but *Elodea* and *Pontederia* can scarcely be considered very significant and possibly have a wider range. If the relative species populations of these 49 genera are studied it will be found that of those in subdivision 3, 8 are predominantly southern, 2 are about equal, and only 1, *Amsinckia*, is predominantly northern. In subdivision 4, 5 genera are predominantly northern, often markedly so as *Eriogonum*, and only 1, *Spirostachys*, is southern. In subdivision 5, 12 of the genera are predominantly northern, 3 are equally distributed, and only 1, *Munroa*, is predominantly southern. In so far as density of species population can be taken to indicate place of origin these figures suggest strongly that there has been a double migration, of southern genera towards the north and vice versa. All the genera are chiefly confined to the western side of the continent, and California is the part of North America most concerned.

In this category the genera belong to 63 families, 32 of which are represented by single genera. The Compositae is very much the largest family with 37 genera, followed by Gramineae with 13 and Leguminosae with 11.

*Category 5. Genera more or less throughout the tropics of both hemispheres.*

The number of genera in this category is 224.

About 30 of these are pan-tropical only by virtue of the exceptionally wide range of one or few species, e.g. *Cocos*, *Canna*, *Flaveria*. *Gynandropsis*, *Lippia*, *Scoparia*. It is impossible to say to what extent they have been spread by human agency.

About 80 are predominantly American, e.g. *Abutilon*, *Psychotria*, *Cassia*, *Croton*, *Eriochloa*, and *Passiflora*. Of the 30 mentioned just above practically all are also in the 80.

A very few, apparently only 3, *Gomphrena*, *Dodonaea*, and *Weinmannia* are chiefly Australian.

20 of the genera in the category, like *Solanum*, *Lobelia*, *Cynanchum*, *Eriocaulon*, and *Aristolochia*, have many temperate species.

It may be noted that the genera with one or more very wide species, such as are frequently common tropical weeds, are practically all centred in the New World.

Most of the 224 genera are represented in temperate S. America only by a few stragglers and are clearly tropical genera with a few temperate outliers. Such are *Cordia*, *Clitoria*, *Erythroxylon*, *Ficus*, *Pogonia*, *Randia*, *Strychnos* and many others.

A very few genera are represented in America by only one or two wide species e.g. *Cynodon* (if native), *Ilysanthes*, *Sorghum*, *Vallisneria*, and *Vigna*.

The genera of the category are contained in 79 families, 44 having 1 genus each only. Here the largest family is the Gramineae with 30 genera, and this followed by the Leguminosae with 26. Compositae are third with 14.

*Category 6. Genera incompletely tropical in distribution.*

The number of genera in this category is 91.

These fall into several subdivisions according to their distribution:

1. American—African genera. The 41 genera of this group include one or two not strictly so confined, but clearly to be included here rather than elsewhere. They include—

*Asclepias*, with one or two pan-tropical species.

*Bartisia*, with extensions into temperate regions.

*Corrigiola*, with a European species.

*Malvastrum*, a difficult and doubtful genus.

*Rheedia*, outside America only in Madagascar.

*Stillingia*, outside America only in Madagascar and Polynesia.

*Rhipsalis*, whose status in Africa is doubtful.

Of the 41, 28 are predominantly American e.g. *Paepalanthus*, *Guarea*, and *Mayaca*: 4 are predominantly African, and 9 are about equally divided.

2. American—African—Asiatic genera. These number 32, including *Urera* which occurs also in Polynesia. Examples are *Pterocarpus*, *Xylopia*, *Mimosa*, and *Dorstenia*.

3. American—Asiatic—Australian genera number 6.

4. American—African—Australian genera number 5.

5. American—Asiatic genera number 6 including *Styrax* and *Phoebe* with European species.

*Centipeda* is a doubtful genus apparently occurring in temperate S. America, tropical Africa, tropical Asia, and tropical Australia.

53 families are represented in the category but no one is very large, Gramineae with 7 heading the list. The Compositae and Leguminosae follow with 6 each. 34 families have single genera only.

Category 7. Genera either pan-temperate or more or less cosmopolitan.

The number of genera in the category is 74.

It should be noted here, and in the next category also, that it is often very difficult in the case of temperate genera to tell whether the occurrence of a genus in a particular region is due to human agency or not. Frequently an arbitrary decision has had to be taken for statistical purposes.

A good many of the genera in this category have numerous tropical species (just as some in category 5 have many temperate species), e.g. *Cyperus*, *Clematis*, *Gnaphalium*, *Senecio*, *Rubus*, and *Euphorbia*. Others, and these particularly include aquatic genera, are nearly or quite cosmopolitan by virtue of one very wide species as in *Hippuris*, *Anagallis*, *Ruppia*, *Limosella*, and others.

In most of the genera the species centre is in the north, sometimes in N. America, as in *Lepidium* only, sometimes in the northern temperate Old World as in *Clematis*, *Avena*, and *Hypericum*, and sometimes throughout the north, as in *Cardamine*, *Ranunculus*, *Juncus*, *Polygonum*, and *Rumex*. Some are centred in one or more parts of the south as *Hydrocotyle*, *Schoenus*, *Oxalis*, *Samolus*, and *Drosera*. In no case is a genus outstandingly numerous in temperate S. America only among the southern regions. Three genera deserve special mention. *Oxalis* actually shows a great American-African affinity and might have been included in category 6. *Drosera* is represented in temperate S. America by two groups of species. one belonging to the section *Rissolis* in S. Brazil &c., and by an American-Australasian section in Fuegia. *Carex* has representatives of all its subgenera in temperate S. America but very unequally and there is no striking peculiarity of species.

The genera of the category belong to 36 families, of which 22 have single genera only. The Gramineae is the largest with 13 genera. Next come Compositae with 6 and Cyperaceae with 5.

Category 8. *Genera incompletely temperate in distribution.*

This, on account of the discontinuity of many of the genera and the various problems it involves, is a very important category and is considered in greater detail than any of the others.

The number of genera in the category is 106.

On the score of endemic species, 73 of these are predominantly northern. 7, namely *Briza*, *Frankenia*, *Lycium*, *Verbena*, *Valeriana*, *Gaultheria*, and *Prosopis* are predominantly southern. 25 are about equally divided and one, *Hypochaeris*, is doubtful, since the American species are often placed in a separate genus *Achyrophorus*. It has been thought best, however, to include it here.

*Briza* ranges through America and is actually most numerous in tropical S. America. *Frankenia* is centred in temperate S. America, the Mediterranean, and Australia, and has species in S. Africa. *Gaultheria* is very closely related to *Pernettya* in category 9 and can hardly be separated. It has most species in tropical S. America and many also in East Asia. *Lycium* is centred in temperate S. America, tropical S. America, and in S. Africa. *Verbena* is centred in temperate S. America, but extends throughout the continent, and the extra-American representation is very slight. *Valeriana* has its population centres in temperate and tropical S. America but is well represented in the north. *Prosopis* is centred in temperate and tropical S. America. It will be seen that in these seven genera there is a very strong tropical American element which is presumably a connecting link between their widely separated temperate range. They have been included in this category because, theoretically at least, they are of chief interest on account of their ranges in temperate regions.

Among the genera which are predominantly northern in species numbers the following occur, in the south, only in temperate S. America:—*Adenocaulon*, *Arenaria*, *Armeria*, *Alnus*, *Alopecurus*, *Antennaria*, *Berberis*, *Catabrosa*, *Centaurea*, *Centunculus*, *Chrysosplenium*, *Draba*, *Empetrum* (and Tristan d'Acunha), *Fragaria*, *Gleditschia*, *Hackelia*, *Hutchinsia*, *Lathyrus*, *Lupinus*, *Linaria*, *Littorella*, *Lonicera*, *Lychnis*, *Myrrhis*, *Muehlenbergia*, *Microcala*, *Milium*, *Oryzopsis*, *Osmorhiza*, *Primula*, *Polemonium*, *Paronychia*, *Phippsia*, *Phleum*, *Pinguicula*, *Ribes*, *Rosa*, *Spiranthes*, *Specularia*, *Sagittaria*, *Sagina*, *Sanicula*, *Satureia*, *Saxifraga*, *Sisyrinchium*, *Solidago*, *Thalictrum*, *Vicia*.

Many of the genera show a marked relation with the Mediterranean region:—*Aira*, *Apium*, *Armeria*, *Centaurea*, *Daucus*, *Eryngium*, *Hordeum*, *Hieracium*, *Lathyrus*, *Linaria*, *Lolium*, *Malva*, *Microcala*, *Micromeria*, *Orobanche*, *Paronychia*, *Phleum*, *Pinguicula*, *Rosa*, *Rubia*, *Stachys*, *Specularia*, *Sagina*, *Satureia*, *Silene*, *Statice*, *Trisetum*, *Teucrium*, *Vicia*. It is worthy of note that in all these, except *Daucus*, *Hypochaeris*, and *Rubia*,

there are N. American species. The only other genus in the category without N. American representation is *Coriaria*.

As regards the distribution of the genera of the category in the northern hemisphere:—

- 86 are in America, Europe, and Asia.
- 4 (mentioned above) are in Europe and Asia only.
- 9 are in North America and Europe only.
- 7 are in North America and Asia only.

Hence out of 106 only 9 are not found in Asia.

The following genera show distinct relationship with the Himalayan region:—*Chrysosplenium*, *Ribes*, *Saxifraga*, *Astragalus*, and *Osmorhiza*. Others show the same in lesser degree. Except for an *Astragalus* in S. Africa these genera are not found below the tropics except in S. America.

As regards the distribution of the genera of the category in the separate parts of the southern hemisphere, all of course occur in S. America, 32 are in S. Africa, 30 in Australia, 23 in New Zealand and/or Polynesia. Among the last *Artemisia*, *Sanicula*, *Silene*, *Thalictrum*, *Verbena*, *Vicia*, and *Erythraea* are not found in New Zealand.

The actual ranges of the genera in the south can be best expressed by using the figures 1, 2, 3, 4 to represent the regions temperate S. America, S. Africa, Australia, and New Zealand and/or Polynesia respectively. The result is:—

1234	2 genera, <i>Myosurus</i> and <i>Triodia</i> .
124	4 "
123	8 "
134	11 "
12	18 "
13	9 "
14	6 "
1	48 "
	<hr/> 106

Thus all the eight possible distributions are represented, the commonest being S. America and S. Africa, and S. America, Australia, and New Zealand and/or Polynesia.

Of the genera found in Australia and/or New Zealand at least 8 are well represented in Eastern Asia. These genera also all occur in N. America.

This category, including as it does a large number of genera which show marked discontinuity between the northern and the southern hemispheres, naturally raises one of the chief problems connected with such ranges, the problem of whether the discontinuity results from a northern movement of southern genera, or from the reverse, or by a double spread from an intermediate point. Whether any facts in the present distribution of genera concerned can be used as definite evidence in favour of one or other views is doubtful. Very careful thought has been given to this

question in the preparation of this article, but so far no method of solving the problem has presented itself. At the same time many of the genera do indicate strongly some *prima facie* probability in one direction or another, and although these cannot be classed as evidence they are of sufficient importance and interest to be cited here.

In 48 of the genera, temperate S. America is the only occurrence south of the tropics. Of these genera 38 are found distributed all over the northern temperate while the remaining 10 are absent from either Western or Eastern Asia or both. They are:—

- Centunculus*. One species only. Absent from East Asia.
- Gleditschia*. A very difficult genus of doubtful statistical value.
- Littorella*. American except for the European species.
- Muehlenbergia*. Very predominantly American with a few species in East Asia.
- Microcala*. Compares with *Centunculus* but has two species.
- Specularia*. Compares closely with the former.
- Osmorhiza*. Seems a clear example of an American-Asiatic genus which has spread southwards.
- Sisyrinchium*. American except for the European species.
- Prosopis*. Suggest movement north. Its relationships are southern.
- Adenocaulon*. Perhaps compares with *Osmorhiza*.

Partly on account of the preponderance of land in the north and partly because of more obscure psychological associations there is a tendency to assume the movement of northern plant groups south rather than the movement of southern groups northward. Humanistically the northern temperate regions form the hub of the wheel from which so much radiates. Botanically there is no such balance against the south. As has been said the arguments here have to be based on indications and not on real evidence, and on indications alone there is every reason to believe that the movements of plants across the equator has been at least a double one, and possibly more from south to north than the reverse. The following are especially good examples of genera which suggest very strongly origin in the south and subsequently migration north.

- Frankenia*. Only Mediterranean in the north. Relationships markedly southern.
- Gaultheria*. Its distribution, see map, strongly suggest a double movement northward. Its very close relation with *Pernettya* supports this.
- Triodia*. In North America and Mediterranean and in all southern regions.
- Verbena*. Great preponderance of species in S. America. One recorded in Hawaii.
- Valeriana*. Great preponderance of species in S. America. Also elsewhere in the south.
- Eryngium*. More widely spread in the south than in the north.
- Mimulus*. Has great species centre in North America but is otherwise chiefly southern. See monograph by Grant, Ann. Missouri Bot. Gard. xi. 1924.
- Euphrasia*. A particularly interesting genus. See Du Rietz, Svensk. Bot. Tid. 25, 1931.
- Myosurus*. Has recently been found in S. Africa. The species are confused. One is said to have the range California, Chile, and New Zealand. Another, North Temperate and Australia.
- Lycium*. Definitely southern on species numbers.

These are some of the most outstanding genera, but almost any one in

the category is well worth careful study in this connexion. Many of them require careful systematic revision.

Further analysis of this category can be conveniently left till after the next, and last, category is considered, but in view of the trans-antarctic dis-

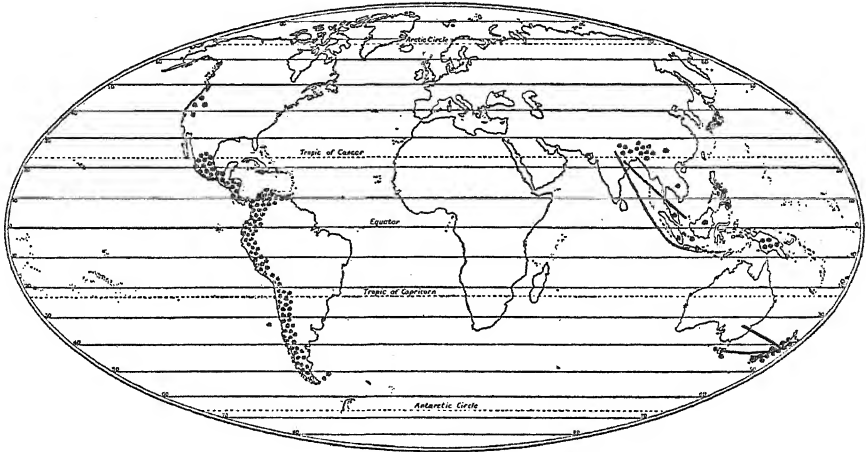


FIG. 2. The distribution of the genera *Gaultheria* and *Pernettya* as taken from the Kew Index. Each dot or line represents a species.

continuities which will be apparent there it is desirable here to summarize the existence of such discontinuity in this category. 11 genera, *Caltha*, *Euphrasia*, *Elatine*, *Gentiana*, *Montia*, *Chenopodium*, *Erythraea*, *Linum*, *Calystegia*, *Gaultheria*, and *Mimulus* occur in S. America, Australia, and New Zealand. 9 genera, *Eryngium*, *Daucus*, *Elymus*, *Glycyrrhiza*, *Sambucus*, *Stellaria*, *Polycarpon*, *Apium*, and *Scutellaria* are found in S. America and Australia. 2 genera, *Deschampsia* and *Coriaria* are found in S. America, New Zealand, and Polynesia. The other four genera with the distribution 14 do not occur in New Zealand.

The genera of the category belong to 42 families. Gramineae comes easily first with 18 genera, twice as many as Compositae which occupies second place. 26 families show one genus each only.

*Category 9. Genera confined entirely or almost entirely to the Southern Hemisphere.*

Number of genera in the category, 53.

The genera vary considerably in reliability. One group, containing 45, consists of genera of whose validity there can be no reasonable doubt or which are supported by detailed studies or monographs. The other group, contains the remaining 7 genera which belong to difficult families where the generic distinctions are unsatisfactory or which for other reasons are better treated as rather dubious. They are *Abrotanella*, *Erechtites*,

*Lagenophora*, *Microseris*, *Vittadinia*, *Trichocline* (Compositae), *Pseudopanax* (Araliaceae), and *Distichlis* (Gramineae).

Most of the genera of group 1 are strictly confined to the southern hemisphere, but the following extend slightly north of the equator:—

*Acaena* in N. America and Hawaii.  
*Cotula* in C. America and the Mediterranean.  
*Calandrinia* in C. and N. America.  
*Calceolaria* in C. America.  
*Fuchsia* in C. America and West Indies.  
*Gunnera* in C. America and Hawaii.  
*Lomatia* in East Asia.  
*Lilaeopsis* in C. and N. America.  
*Mesembryanthemum* in N. America and Mediterranean.  
*Muehlenbeckia* in C. and N. America.  
*Nertera* in Asia.  
*Oreobolus* in Hawaii.  
*Oreomyrrhis* in C. America.  
*Pernettya* in C. America.  
*Roupala* in C. America and West Indies.  
*Santalum* in Hawaii.  
*Uncinia* in C. America.  
*Villaresia* in C. America.

Similarly of the genera in group 2:—

*Erechtites* in C. and N. America.  
*Microseris* in N. America.  
*Vittadinia* in Hawaii.  
*Distichlis* in C. and N. America.  
*Lagenophora* in Hawaii.

It will be noted that only two of all these are in Asia. The rest are in Central America, North America, or Hawaii.

*Acaena*, *Azorella*, *Colobanthus*, *Cotula*, *Coprosma*, *Lagenophora*, *Nertera*, and *Uncinia* have species on one or more of the small 'South Atlantic' oceanic islands.

Analysed according to their southern distributions the genera of the category give the following results. The numbers 1, 2, 3, 4 refer to S. America, S. Africa, Australia, and New Zealand and/or Polynesia respectively:—

- 134 *Azorella*, *Abrotanella*, *Aristotelia*, *Colobanthus*, *Coprosma*, *Carpaea*, *Cordyline*, *Drapetes*, *Drimys*, *Discaria*, *Erechtites*, *Lagenophora*, *Libertia*, *Lomatia* (not N.Z.), *Luzuriaga*, *Lilaeopsis*, *Microseris*, *Muehlenbeckia*, *Nothofagus*, *Nertera*, *Oursia*, *Oreobolus*, *Oreomyrrhis*, *Pernettya*, *Phyllachne*, *Pratia*, *Roupala* (not N.Z.), *Santalum*, *Selliera*, *Uncinia*.  
 13 *Calandrinia*, *Embothrium*, *Eucryphia*, *Leptocarpus*, *Villaresia*, *Vittadinia*, *Trichocline*, *Distichlis*.  
 14 *Calceolaria*, *Donatia*, *Fuchsia*, *Gaimardia*, *Griselinia*, *Laurelia*, *Marsippospermum*, *Pseudopanax*, *Rostkovia*, *Tetrachondra*, *Hebe*.  
 1234 *Acaena*, *Gunnera*, *Mesembryanthemum*.  
 123 *Cotula*.  
 12 and 124 (the remaining possibilities (none)).

Of the genera with the range 14 only *Calceolaria* and *Fuchsia* reach across the equator, and these only in Central America. It will be remembered that of the genera in category 8 only one had a similar southern



distribution, *Coriaria*. Hence, it would appear that it is very rarely that genera, in the south discontinuous between S. America and New Zealand, reach the northern hemisphere. As will be seen, northern ranges are much more common in genera which have 13 or 134 distributions in the south, namely, those which occur in Australia.

Of all the genera of the category only 4 are found in S. Africa. The genera of the category belong to 35 families, the actual figures being as follows:—

7 genera	Compositae.
3 "	Scrophulariaceae, Umbelliferae, Proteaceae, Cyperaceae.
2 "	Stylidiaceae, Rubiaceae, Liliaceae, Juncaceae.
1 "	Gramineae, Boraginaceae, Caryophyllaceae, Iridaceae, Polygonaceae, Rosaceae, Portulacaceae, Campanulaceae, Onagraceae, Santalaceae, Monimiaceae, Restionaceae, Rhamnaceae, Ericaceae, Thymeleaceae, Araliaceae, Winteraceae, Eucryphiaceae, Centrolepidaceae, Cornaceae, Aizoaceae, Fagaceae, Goodeniaceae, Olacaceae, Haloragidaceae, Elaeocarpaceae.

Now that all the categories have been dealt with it is possible to give a more complete analysis of the distributions of the more temperate genera which they contain. These are included in categories 1, 4, 7, 8, 9. The analysis may be made, in a way which has been already illustrated, by giving numbers to the different parts of the temperate regions and dividing the genera into groups according to the figures of their ranges. For this purpose the seven main temperate divisions, North America, Europe and West Asia, Eastern Asia, South America, South Africa, Australia and New Zealand and Polynesia are given the numbers 1 to 7 respectively. Allowing for the fact that only genera which occur in division 4, temperate S. America, are considered here, there are 64 possible distributions ranging from 4 (genera endemic to temperate S. America) to 1234567 (pan-temperate genera). Of these 64 possibilities, only 28 actually occur, and 36 do not.

The complete analysis giving distributional formula and number of genera in each, in the order of the latter, is as follows:—

Number of genera.	Formula.	Number of genera.	Formula.
284	4	4	123457
74	1234567	2	146
37	1234	2	2345
26	467	2	13467
17	12345	2	124567
16	14		
11	47	1	1246
9	123467	1	2346
7	123456	1	2347
7	2346	1	4567
6	46	1	2456
5	124	1	12456
5	134	1	12457
5	12347	1	14567
4	1467		

The 36 formulae which do not occur are:

24, 34, 45.

145, 147, 234, 245, 246, 345, 346, 347, 456, 457, 247.

1247, 1346, 1347, 1456, 1457, 1345, 2457, 2467, 3456, 3457, 3467, 1245.

13456, 13457, 12467, 23456, 23457, 24567, 23467, 34567.

134567, 234567.

*Table of Families and Categories.*

(See p. 699).

Family.	Categories.									Remarks.	
	1.	2.	3.	4.	5.	6.	7.	8.	9.	No. of gen.	No. of cats.
Acanthaceae . . .	—	1	2	4	5	2	—	—	—	14	5
Aextoxicaceae . . .	1	—	—	—	—	—	—	—	—	1	1
Aizoaceae . . . . .	—	—	—	—	1	—	—	—	1	2	2
Alismataceae . . . . .	—	—	—	—	—	1	—	1	—	2	2
Amarantaceae . . . . .	2	3	—	4	4	—	—	—	—	13	4
Amaryllidaceae . . . . .	9	5	2	3	1	—	—	—	—	20	5
Ampelidaceae . . . . .	—	—	—	—	2	—	—	—	—	2	1
Anacardiaceae . . . . .	1	2	3	1	—	—	—	—	—	7	4
Anonaceae . . . . .	—	1	1	—	1	1	—	—	—	4	4
Apocynaceae . . . . .	2	3	4	6	1	1	—	—	—	17	6
Araceae . . . . .	1	4	5	1	1	—	—	—	—	12	5
Araliaceae . . . . .	—	—	1	—	—	1	—	—	1	3	3
Aristolochiaceae . . . . .	1	1	—	—	1	—	—	—	—	3	3
Asclepiadaceae . . . . .	2	14	3	5	3	2	—	—	—	29	6
Basellaceae . . . . .	—	—	1	—	—	—	—	—	—	1	1
Berberidaceae . . . . .	—	—	—	—	—	—	—	1	—	1	1
Betulaceae . . . . .	—	—	—	—	—	—	—	1	—	1	1
Bignoniaceae . . . . .	3	5	11	1	1	—	—	—	—	21	5
Bixaceae . . . . .	—	—	—	—	1	—	—	—	—	1	1
Bombacaceae . . . . .	—	—	1	—	2	1	—	—	—	4	3
Boraginaceae . . . . .	8	2	1	6	4	—	1	2	1	25	8
Bromeliaceae . . . . .	7	8	5	1	—	—	—	—	—	21	4
Burmanniaceae . . . . .	1	—	—	—	1	—	—	—	—	2	2
Butomaceae . . . . .	1	2	—	—	—	—	—	—	—	3	2
Cactaceae . . . . .	6	1	3	3	—	1	—	—	—	14	5
Callitrichaceae . . . . .	—	—	—	—	—	—	1	—	—	1	1
Calyceraceae . . . . .	1	2	1	—	—	—	—	—	—	4	3
Campanulaceae . . . . .	2	—	2	1	2	—	—	1	1	9	6
Cannaceae . . . . .	—	—	—	—	1	—	—	—	—	1	1
Capparidaceae . . . . .	1	1	—	—	4	—	—	—	—	6	3
Caprifoliaceae . . . . .	—	—	—	—	—	—	—	2	—	2	1
Caricaceae . . . . .	—	—	1	1	—	—	—	—	—	2	2
Caryocaraceae . . . . .	—	—	1	—	—	—	—	—	—	1	1
Caryophyllaceae . . . . .	5	1	1	—	2	1	2	7	1	20	8
Celastraceae . . . . .	1	—	1	—	—	—	—	—	—	2	2
Centrolepidaceae . . . . .	—	—	—	—	—	—	—	—	1	1	1
Ceratophyllaceae . . . . .	—	—	—	—	—	—	1	—	—	1	1
Chenopodiaceae . . . . .	2	1	—	2	—	—	4	1	—	10	5
Chloranthaceae . . . . .	—	—	1	—	—	—	—	—	—	1	1
Cistaceae . . . . .	—	—	—	1	—	—	—	—	—	1	1
Cochlospermaceae . . . . .	—	—	—	—	1	—	—	—	—	1	1
Combretaceae . . . . .	—	—	—	—	1	1	—	—	—	2	2
Commelinaceae . . . . .	—	—	1	2	3	—	—	—	—	6	3
Compositae . . . . .	56	28	17	37	14	6	9	7	7	180	9
Convolvulaceae . . . . .	—	—	—	—	7	—	1	1	—	9	3
Coriariaceae . . . . .	—	—	—	—	—	—	—	1	—	1	1
Cornaceae . . . . .	—	—	—	—	—	—	—	—	1	1	1
Crassulaceae . . . . .	—	—	—	—	1	1	—	—	—	2	2

Family.	Categories.									Remarks.	
	1.	2.	3.	4.	5.	6.	7.	8.	9.	No. of gen.	No. of cats.
Cruciferae . . . . .	8	5	—	1	—	—	4	3	—	21	5
Cucurbitaceae . . . . .	1	3	2	4	2	—	—	—	—	12	5
Cunoniaceae . . . . .	1	1	—	—	1	—	—	—	—	3	3
Cyperaceae . . . . .	—	1	1	1	8	2	5	—	3	21	7
Dioscoreaceae . . . . .	1	—	—	—	1	—	—	—	—	2	2
Droseraceae . . . . .	—	—	—	—	—	—	1	—	—	1	1
Ebenaceae . . . . .	1	—	—	—	2	—	—	—	—	3	2
Elaeocarpaceae . . . . .	1	1	—	—	—	—	—	—	1	3	3
Elatinaceae . . . . .	—	—	—	—	—	—	—	1	—	1	1
Empetraceae . . . . .	—	—	—	—	—	—	—	1	—	1	1
Epacridaceae . . . . .	1	—	—	—	—	—	—	—	—	1	1
Ericaceae . . . . .	—	1	—	1	—	—	—	1	1	4	4
Eriocaulaceae . . . . .	—	2	—	—	1	2	—	—	—	5	3
Erythroxylaceae . . . . .	—	—	—	—	1	—	—	—	—	1	1
Eucryphiaceae . . . . .	—	—	—	—	—	—	—	—	1	1	1
Euphorbiaceae . . . . .	3	3	3	5	6	5	1	—	—	26	7
Fagaceae . . . . .	—	—	—	—	—	—	—	—	1	1	1
Flacourtiaceae . . . . .	3	—	3	—	1	1	—	—	—	8	4
Frankeniaceae . . . . .	1	1	—	—	—	—	—	1	—	3	3
Gentianaceae . . . . .	—	2	1	1	1	—	—	3	—	8	5
Geraniaceae . . . . .	1	2	—	—	—	—	1	—	—	4	3
Gesneriaceae . . . . .	3	—	5	—	—	—	—	—	—	8	2
Goodeniaceae . . . . .	—	—	—	—	—	—	—	—	1	1	1
Gramineae . . . . .	13	6	5	13	30	7	13	18	1	106	9
Guttiferae . . . . .	—	1	—	—	—	1	1	—	—	3	3
Haloragidaceae . . . . .	—	—	—	—	—	—	1	—	1	2	2
Hippuridaceae . . . . .	—	—	—	—	—	—	1	—	—	1	1
Hydnoraceae . . . . .	1	—	—	—	—	—	—	—	—	1	1
Hydrocharitaceae . . . . .	—	1	—	2	2	—	—	—	—	5	3
Hydrophyllaceae . . . . .	—	—	—	2	1	—	—	—	—	3	2
Icacinaeae . . . . .	—	1	—	—	—	—	—	—	—	1	1
Ilicineae . . . . .	—	—	—	—	—	1	—	—	—	1	1
Iridaceae . . . . .	3	4	4	3	—	1	—	1	1	17	7
Juncaceae . . . . .	3	1	—	—	—	—	2	—	2	8	4
Labiatae . . . . .	5	5	—	3	3	—	—	6	—	22	5
Lacistemaceae . . . . .	—	1	—	—	—	—	—	—	—	1	1
Lactoridaceae . . . . .	1	—	—	—	—	—	—	—	—	1	1
Lardizabalaceae . . . . .	1	1	—	—	—	—	—	—	—	2	2
Lauraceae . . . . .	1	—	2	—	1	2	—	—	—	6	4
Lecythidaceae . . . . .	—	—	1	—	—	—	—	—	—	1	1
Leguminosae . . . . .	10	15	16	11	26	6	1	8	—	93	8
Lemnaceae . . . . .	—	—	—	1	—	—	2	—	—	3	2
Lentibulariaceae . . . . .	—	—	—	—	1	1	—	1	—	3	3
Liliaceae . . . . .	16	2	—	2	1	3	—	—	2	26	6
Linaceae . . . . .	—	—	—	—	—	—	—	1	—	1	1
Loasaceae . . . . .	2	1	1	1	—	—	—	—	—	5	4
Loganiaceae . . . . .	—	1	—	1	1	1	—	—	—	4	4
Loranthaceae . . . . .	1	1	1	1	1	—	—	—	—	5	5
Lythraceae . . . . .	—	2	1	1	—	—	1	—	—	5	4
Malesherbiaceae . . . . .	1	1	—	—	—	—	—	—	—	2	2
Malpighiaceae . . . . .	3	7	4	5	—	1	—	—	—	20	5
Malvaceae . . . . .	3	2	2	2	5	4	—	1	—	19	7
Marantaceae . . . . .	—	—	3	—	—	1	—	—	—	4	2
Martyniaceae . . . . .	—	1	—	1	—	—	—	—	—	2	2
Mayaceae . . . . .	—	—	—	—	—	1	—	—	—	1	1
Melastomaceae . . . . .	—	—	7	—	—	—	—	—	—	7	1
Meliaceae . . . . .	—	1	1	—	1	1	—	—	—	4	4
Menispermaceae . . . . .	—	—	1	—	—	—	—	—	—	1	1
Monimiaceae . . . . .	2	—	1	—	—	—	—	—	1	4	3

Family.	Categories.									Remarks.	
	1.	2.	3.	4.	5.	6.	7.	8.	9.	No. of gen.	No. of cats.
Moraceae . . . .	1	—	4	—	2	2	—	—	—	9	4
Musaceae . . . .	—	—	1	—	—	—	—	—	—	1	1
Myrsinaceae . . . .	—	—	1	—	1	—	—	—	—	2	2
Myrtaceae . . . .	3	5	5	1	2	—	—	—	—	16	5
Myzodendraceae . . . .	2	—	—	—	—	—	—	—	—	2	1
Naiadaceae . . . .	—	—	—	—	—	—	1	—	—	1	1
Nolanaceae . . . .	1	4	—	—	—	—	—	—	—	5	2
Nyctaginaceae . . . .	1	4	1	3	2	—	—	—	—	11	5
Nymphaeaceae . . . .	—	1	—	1	1	—	—	—	—	3	3
Ochnaceae . . . .	—	—	—	—	1	—	—	—	—	1	1
Olaceae . . . .	—	—	—	—	1	1	—	—	1	3	3
Oleaceae . . . .	—	—	—	—	—	2	—	—	—	2	1
Onagraceae . . . .	1	1	—	4	1	—	1	—	1	9	6
Opiliaceae . . . .	—	—	1	—	—	—	—	—	—	1	1
Orchidaceae . . . .	3	5	21	4	5	2	—	1	—	41	7
Orobanchaceae . . . .	—	—	—	—	—	—	—	1	—	1	1
Oxalidaceae . . . .	—	1	—	—	—	—	1	—	—	2	2
Palmaceae . . . .	2	3	4	—	1	—	—	—	—	10	4
Papaveraceae . . . .	—	—	1	1	—	—	—	—	—	2	2
Passifloraceae . . . .	—	1	—	—	1	—	—	—	—	2	2
Phytolaccaceae . . . .	1	1	4	1	—	2	—	—	—	9	5
Piperaceae . . . .	—	—	—	—	2	—	—	—	—	2	1
Plantaginaceae . . . .	—	—	—	—	—	—	1	1	—	2	2
Plumbaginaceae . . . .	—	—	—	—	1	—	—	2	—	3	2
Podostemaceae . . . .	—	—	—	—	—	1	—	—	—	1	1
Polemoniaceae . . . .	—	—	—	2	—	—	—	1	—	3	2
Polygalaceae . . . .	1	—	—	1	1	1	—	—	—	4	4
Polygonaceae . . . .	—	—	3	3	—	—	2	—	1	9	4
Pontederiaceae . . . .	—	1	—	1	—	2	—	—	—	4	3
Portulacaceae . . . .	4	1	—	—	1	1	—	1	1	9	6
Potamogetonaceae . . . .	—	—	—	—	—	—	3	1	—	4	2
Primulaceae . . . .	1	—	—	—	—	—	3	2	—	6	3
Proteaceae . . . .	1	—	—	—	—	—	—	—	3	4	2
Ranunculaceae . . . .	2	—	—	—	—	—	2	4	—	8	3
Restionaceae . . . .	—	—	—	—	—	—	—	—	1	1	1
Rhamnaceae . . . .	—	3	2	1	1	1	—	—	1	9	6
Rosaceae . . . .	1	4	—	—	—	—	2	2	1	10	5
Rubiaceae . . . .	2	7	17	2	5	2	1	1	2	39	9
Rutaceae . . . .	1	1	2	1	1	—	—	—	—	6	5
Salicaceae . . . .	—	—	—	—	—	—	—	1	—	1	1
Santalaceae . . . .	3	3	—	—	—	—	—	—	1	7	3
Sapindaceae . . . .	3	6	3	1	4	2	—	—	—	19	6
Sapotaceae . . . .	—	—	3	1	2	—	—	—	—	6	3
Saxifragaceae . . . .	6	1	—	1	—	1	—	3	—	12	5
Scheuchzeriaceae . . . .	1	1	—	—	—	—	1	—	—	3	3
Scrophulariaceae . . . .	4	—	3	2	5	2	1	3	3	23	8
Simarubaceae . . . .	—	—	1	2	—	—	—	—	—	3	2
Solanaceae . . . .	14	7	8	4	5	—	—	1	—	39	6
Sterculiaceae . . . .	—	—	1	1	2	1	—	—	—	5	4
Stylidiaceae . . . .	—	—	—	—	—	—	—	—	2	2	1
Styraceae . . . .	—	—	—	—	—	1	—	—	—	1	1
Symplocaceae . . . .	—	—	—	—	—	1	—	—	—	1	1
Thymeleaceae . . . .	—	1	1	—	—	—	—	—	1	3	3
Tiliaceae . . . .	—	—	1	—	2	—	—	—	—	3	2
Tropaeolaceae . . . .	1	—	1	—	—	—	—	—	—	2	2
Turneraceae . . . .	—	—	—	—	1	1	—	—	—	2	2
Typhaceae . . . .	—	—	—	—	—	—	1	—	—	1	1
Ulmaceae . . . .	—	1	—	—	2	—	—	—	—	3	2
Umbelliferae . . . .	9	1	—	1	—	—	1	6	3	21	6

Family.	Categories.									Remarks.	
	1.	2.	3.	4.	5.	6.	7.	8.	9.	No. of gen.	No. of cats.
Urticaceae . . . .	—	—	1	—	1	1	2	—	—	5	4
Vacciniaceae . . . .	—	—	—	—	—	1	—	—	—	1	1
Valerianaceae . . . .	—	1	1	1	—	—	—	1	—	4	4
Velloziaceae . . . .	—	—	—	—	—	1	—	—	—	1	1
Verbenaceae . . . .	6	3	2	1	5	1	—	1	—	19	7
Violaceae . . . .	1	1	1	—	1	—	1	—	—	5	5
Vochysiaceae . . . .	—	1	1	—	—	—	—	—	—	2	2
Winteraceae . . . .	—	—	—	—	—	—	—	—	1	1	1
Xyridaceae . . . .	—	—	—	—	1	—	—	—	—	1	1
Zingiberaceae . . . .	—	—	—	—	1	—	—	—	—	1	1
Zygophyllaceae . . . .	4	1	—	2	—	1	—	—	—	8	4
	284	233	229	184	224	91	74	106	53	1478	

*Comparison with the Other Temperate Southern Floras.*

Attention was drawn in the early part of the introduction to the desirability of making some such analysis of the temperate S. American flora as has just been completed in order to facilitate a comparison between that flora and the other floras of the southern temperate zone. There follows now a short comparison of the temperate S. American and S. African floras together with a few remarks on a comparison of the temperate S. American and temperate Australian floras.

The necessary statistics of the S. African flora are easily obtained from Phillip's 'Genera of South African Plants'. If a summary of the contents of this work is made, the most salient figures relating to native plants for the two floras is as follows:—

South Africa.		South America.	
173	number of families	177	
1492	number of genera	1478	
c. 15375	number of species	c. 12250	
72	families with endemic genera	77	
488	endemic genera	284	
190	monotypic endemic genera	c. 150	
3186	species in endemic genera	c. 865	

In the S. African flora there are 6 endemic families with about 90 species: in the S. American flora there are 2 endemic families with 12 or 15 species.

It will be seen that the figures in this table are, for the two floras, either very similar or very dissimilar. Figures 1, 2, 4, and, to a lesser degree, 6, are much alike. The number of species, the number of endemic genera, and the number of species in these genera are on the other hand, very different, and in each case S. Africa has a big advantage. If the respective areas are considered, the difference is still more striking. In round figures temperate S. America is about  $2\frac{1}{2}$  times as large as S. Africa. This being so the flora of the former, in order to compare exactly with the

flora of the latter should contain 3,700 genera, and 38,500 species of which 1,220 genera, containing 8,000 species, should be endemic. Conversely, if the S. African flora was only, comparatively, as large as that of S. America, it would contain 600 genera with less than 5,000 species, of which 117 genera with 350 species would be endemic. It must be remembered, however, in making this comparison, that areas of widely different latitude are concerned.

It is clear from this that the S. American flora is considerably less rich than that of S. Africa, although the actual numbers of families and genera are much the same. The difference lies rather in the greater density of species and the greater range of endemic forms as well as in the higher generic species numbers of the latter. For example, the average number of species per genus in S. Africa is 10.3 as compared with 8.2 in S. America, a small but significant difference. The proportion, too, of endemic genera in S. Africa is 31 per cent as opposed to just under 20 per cent. The species number of endemic genera is 6.5 as against 3, and this again is an important distinction.

Although the numbers of the families in the two floras are so similar, the families are by no means exactly the same in both. Of the 173 families in S. Africa 37 are not represented in S. America, and of the 177 in S. America 39 are not represented in S. Africa. Two are doubtful. These are all mostly very small families, the largest being the Bromeliaceae and Tropaeolaceae in S. America and the Bruniaceae in S. Africa. If the families which have endemic genera in their respective floras are considered only, S. Africa has 29 not in S. America, and S. America 33 not in S. Africa.

Another interesting point in the comparison is that of the genera which, while occurring in the temperate S. American flora, are confined to the American continent, with the genera occurring in S. Africa and confined to the continent of Africa, Arabia, and Madagascar:

In the temperate S. American flora there are:

284 endemic genera,  
233 genera confined to S. America,  
229 genera confined to Tropical and S. America,  
184 genera ranging over America as a whole.

Total: 930 genera or about 63 per cent.

In the S. African flora there are:

488 endemic genera,  
228 genera confined to Africa and Arabia,  
44 genera confined to Africa and Madagascar, &c.

Total: 760 genera or 51 per cent.

Thus there seems to be a difference in the type of distribution of the bulk of the genera in the two. In the S. American flora there are fewer rigidly

endemic genera and more scattered widely over the continent, while in S. Africa the reverse is the case.

But the great distinction between the two floras is really the absence from S. America of the huge endemic or almost endemic genera which form such a feature in S. Africa. The largest genus in the former is *Senecio* with 446 species. This is certainly a very large species number, but the genus is cosmopolitan and has almost equally large species numbers in several other regions. Next to *Senecio* comes *Adesmia* with 260 species, but this genus ranges into tropical America. The third genus is *Oxalis* with 205 species, but this again is a wide genus, although S. America generally is its chief centre. Another great mass of species is in S. Africa. Then there are a dozen other genera with over 100 species each and 38 with over 50, but of these, *Nassauvia*, with 62 species, is the only one strictly endemic to temperate S. America.

In S. Africa, on the other hand, there are two genera, *Erica* and *Mesembryanthemum*, each with over 500 species. Both of these are almost endemic in the sense that very few species extend outside the area. Then come two genera with over 200 each and fifteen with about 100 or more. There are 3 strictly endemic genera with more than 100 species and 6 with 50 or more.

These results may be given in tabular form :

S. America :

Species in the 50 largest genera . . .	4723
Endemic genera in these 50 . . .	1
Number of species in this genus . . .	62
Average species number in the largest genera	93

S. Africa :

Species in the 50 largest genera . . .	5705
Endemic genera in these 50 . . .	9
Number of species in these genera . . .	651
Average species number in the largest genera	113

It may be noted that the figures for S. America are largely derived from the Kew Index, while those of Phillips for S. Africa are apparently more critical estimates. Had the Kew Index been used in both cases the numbers for S. Africa would have been in general considerably higher. For example, the figure 235 for *Senecio* would have been 297.

Another point which the short table does not make clear is that of the 50 largest genera in S. America 22 are genera with very wide distributions, almost world-wide, but of the 50 largest genera in S. Africa less than a dozen are wide, and even these are for the most part less wide than those of S. America. Only three genera, *Oxalis*, *Senecio*, and *Ipomoea*, appear in both lists.

A comparison of the flora of temperate S. America with that of temperate Australia such as has just been made with S. Africa is very much

more difficult. There is no recent general survey of the Australian flora and it would involve, for temperate Australia, a similar compilation as has been done for S. America, and that could only be undertaken in another memoir. At the same time there are certain isolated facts regarding the constitution of the Australian flora which indicate roughly what the result of such a comparison would be. Mueller's 'Census of Australian Plants' published in 1889 gives, for temperate Australia, some 1,000 genera and 8,000 species. Allowing for the enormous increase in knowledge of the floras since that date and for the fact that the flora contains many very large genera such as *Acacia* and *Eucalyptus*, and remembering the marked representation of such families as the Myrtaceae, Proteaceae, Stylidiaceae, and Goodeniaceae, it is clear that the flora as a whole has, despite the difference in area, much greater resemblance in type to that of S. Africa than to that of temperate S. America. In short, there is good reason to believe that much of what has been said for S. Africa would apply also for Australia, and that a comparison would show much the same features. Of the three southern temperate floras, it may therefore be said that those of S. Africa and Australia resemble each other much more closely than either of them resembles that of temperate S. America, and that their differences from this flora are of very much the same kinds.

#### SUMMARY AND CONCLUSIONS.

The outstanding characteristics of the flora of temperate S. America, as revealed by the foregoing analysis and by a comparison with other temperate southern floras, may be summarized as follows:

1. The comparative richness of the flora, taking into account its area, is considerably less than that of S. Africa and, as far as can be estimated, than that of Australia.

2. A very high proportion of the species, probably some 90 per cent., are endemic.

3. A much smaller proportion of the genera, about 20 per cent., are endemic.

4. There are only two endemic families, containing 3 genera and about 15 species.

5. There are no very large strictly endemic genera.

6. The representation of the Compositae, 180 genera and 2,000 species is remarkably great.

7. The Gramineae and Leguminosae are also very strongly represented.

8. The *Solanineae*, and especially the Solanaceae, are particularly well represented.

9. The floristic peculiarities of the flora are not comparable with those of other southern floras.



10. There is a strong relation with the flora of N. America.
11. There is a strong relation with the Mediterranean flora.
12. There is a strong relation with the floras of tropical and S. Africa.
13. There is a strong relation with the flora of Australasia.
14. There is a small but notable relation with the Himalayan flora.
15. There is no marked demarkation between the flora of temperate S. America and the floras of regions of the continent farther north.
16. There is far less relation with the flora of the West Indies than with the floras of other parts of the continent.
17. In many genera the species of temperate S. America are the only species found south of the tropics.
18. Many of the genera have a wide distribution in the northern temperate regions.
19. Many genera of tropical American affinity are represented in the flora by outlying species, and the maximum species centre of many of the genera is in tropical America.
20. Very few families, and these all small, are represented only by endemic genera.

Some of these points have been touched upon already, but others require further consideration.

Point 2, the number of endemic species, is probably not very significant. The average range or area of an angiosperm species over the world has not, so far as the writer is aware, been estimated. It must, however, be conceded that such an average area is comparatively limited. Very few species, except perhaps in the northern temperate, are found native to more than one continent or subcontinent. The great majority have a much narrower range. If the region under discussion is greater than the average species range it therefore follows that the proportion of endemic species will tend to be very high, and there is reason to believe that temperate S. America is of such a size.

Point 3, the proportion of endemic genera, is not susceptible to the same kind of argument because the genus is a much more variable quantity and its range is the combined range of its constituent species. The figure, for temperate S. America, about 20 per cent., is significant here chiefly in comparison with the other two temperate southern floras where the value is much higher. Points 4 and 5 draw attention to other directions in which S. America is at a disadvantage when compared with S. Africa and Australia.

Points 10, 11, 12, 15, 18 are all indications of one of the most interesting features of the S. American flora, the absence of any marked latitudinal limitation. Floristically the segregation of that part of the continent south of the tropic of Capricorn is quite insignificant. The flora merges gradually into the tropical flora, and this in turn merges into the

northern flora. In addition there is almost every kind of distribution between the north and the south. Many genera are found in south, central, and north, others in north and south, others in central and south, and, although not considered here, there are some which range through north and central. But the marked north-south relation of much of the flora is not confined to N. America, and many genera range throughout the northern hemisphere. In numerous cases, which are very important, the temperate S. American species are the only ones south of the tropics. There is also a very marked relation with the flora of the Mediterranean region, and a very much smaller, but even more remarkable, relation with the Himalayan region. These facts cannot be discussed further here, but they are clearly bound up with the peculiar topography and geological history of the American continent. Point 16 seems to indicate clearly the predominantly western path by which much of this wide latitudinal dissemination has taken place.

Points 12 and 13 illustrate what may be looked upon as the second outstanding feature of the flora, its relationship with other southern floras. It should be observed that the relation with Africa is chiefly through tropical genera, which are represented in the temperate S. American flora generally by a few stragglers, but there is also a notable relation with S. Africa in such genera as *Oxalis* and *Asclepias*. With Australia and New Zealand the reverse is the case and the relation, which is one of the best known facts of plant geography, concerns temperate or warm-temperate genera almost entirely. This relation is undoubtedly of very great theoretical interest but its extent must not, on that account, be unduly magnified. The number of genera and species involved is, compared with the floras as a whole, small. In connexion with these genera attention should be drawn to the fact that several are found in Hawaii, and this is often their only occurrence north of the equator.

Point 8 recalls what is practically the only feature of note in the floristic peculiarity of the flora. It illustrates also that there is nothing in the flora comparable with the great numbers of Proteaceae, Ericaceae, Rutaceae, and Monocotyledons in the S. African flora or with the Myrtaceae, Mimosoideae, and Proteaceae of Australia. It is true that these groups are represented, but by very small numbers only. It is also true that there are in temperate S. America many members of the Cactaceae and Bromeliaceae, and that these are characteristic American families, but they are quite definitely tropical families and do not compare with the warm temperate groups mentioned for the other floras. Of these there seems in America very little trace. Even with the *Solanineae* many members of the order have very little representation here, and in any case the order is separated from others on characters which are often more academic than phylogenetic.

Points 7 and 8, referring to the abundance of Composites, Grasses, and Leguminosae, are of considerable interest, but since all three families are very widespread their presence does not give any very special floristic facies to the flora. Moreover, all three are very homogeneous families, and classification and the elucidation of relationships within such families is always difficult. For instance, among the Composites the *Mutisieae* are very fully, indeed almost completely, represented, but it is difficult to say how far the characters used to differentiate this group are really of phylogenetic value. The numbers of grasses may well be due to particularly wide areas of suitable climatic conditions, and the presence of the western mountains probably accounts to some extent for the numbers in the other two families.



# Selection in the Production of the Ever-sporting Stocks.

BY

R. A. FISHER, F.R.S.

With one Diagram in the Text.

## I. *Winge's Theory of Doubleness.*

THE problem of doubleness in stocks, which had been the subject of genetic work at Cambridge from almost the beginning of the century, has been recently cleared up by Winge (O. Winge, 1931, 'The inheritance of double flowers and other characters in *Matthiola*'. *Zeitschrift für Züchtung, Reihe a Pflanzenzüchtung*, xvii. 118-35), by means of extensive experiments of his own, which he finds confirmed by certain unexplained exceptional plants reported by Miss E. R. Saunders as early as 1911.

On Winge's theory the double is a recessive mutant which, since the double flowers are sterile, both as pollen and ovule parent, acts as a lethal. In the ever-sporting races it is balanced by a closely linked pollen lethal. The ever-sporting singles, from which the doubles are derived generation after generation, are thus heterozygous for two closely linked lethals carried in opposite chromosomes. Apart from rare recombinations, the pollen all contains the gene for doubleness, while the ovules are of two kinds, one containing the gene for doubleness, and the other the pollen lethal. Consequently there are produced in each generation nearly half doubles, free from the pollen lethal, and nearly half singles carrying this lethal like their parents. The pollen lethal acts, not only on the gamete, but has also a debilitating effect on the heterozygote; the singles of ever-sporting lines are for this reason relatively weakly plants, compared with the doubles of the same lines, and with singles obtained by out-crossing. Their frequency also shows regularly some slight deficiency compared with the doubles in the same families. This inequality in the numbers surviving to be classified led, for many years, to an elaboration of hypotheses aimed at explaining, by the interaction of two or more factors, the aberrant and irregular ratios observed.

In addition to the offspring produced, as explained above, by

non-crossover gametes, there should, on Winge's hypothesis, be formed a small proportion of normal pollen, which, with the two common types of ovule, will produce two types of single plant bearing either the pollen lethal, or the recessive gene for doubleness, but not both. The second type will also be produced from cross-over ovules. From the progeny of such plants both of these recessive factors will be steadily and automatically eliminated. The pollen lethal could of course only survive in the female line, and even here will be at a disadvantage owing to its debilitating effect. The doubling factor can survive both in pollen and ovules, but since the double plants themselves must be without progeny, the power of throwing doubles could only be retained in the strain by planting different progenies separately, and, in each generation, taking seed only from those singles which had appeared in the same families as doubles. Since this precaution is not needed in the ever-sporting strains of stock, it would certainly not have been taken; and the progeny descended from such cross-over plants, throwing, as long as it was retained, an increasing proportion of singles, would be certainly rejected sooner or later as contaminated seed.

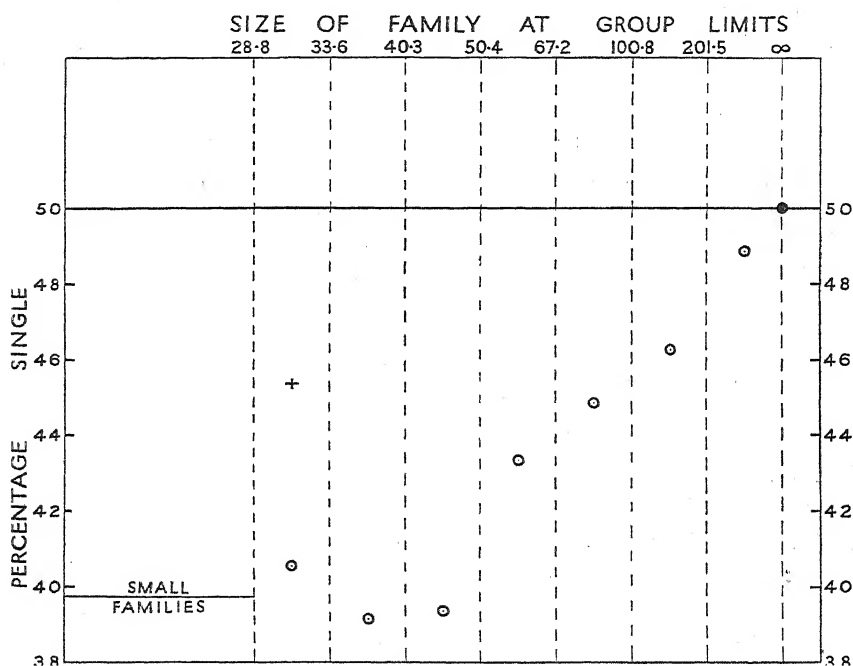
One cross-over plant, from which the pollen lethal had been eliminated, was fortunately used as a parent by Winge, who raised 186 plants from it by self-fertilization; of these 139 were single, and 47 double. Evidently, it differed sharply from the sister plants which gave, as usual, a slight excess of doubles. It was also evidently much more fertile than they were. Only a quarter of the offspring are doubles, and we should therefore expect a second quarter to be pure-breeding singles. Actually, two of the single offspring bred from gave respectively progenies of 945 and 314 plants, all single, while others gave large progenies very closely in the ratio 3:1.

In addition to this well-established case of his own, Winge points out that a probably similar family was reported by E. R. Saunders (1911), ('Further experiments on the inheritance of "doubleness" and other characters in stocks', *Journal of Genetics*, i. 303-76). Of the 87 self-bred families of the Glabrous-red race (Table III, p. 372), the 86th has 23 singles and 8 doubles, numbers strongly suggestive of a 3:1 ratio, and very aberrant from the excess of doubles usually found, especially in the smaller families. Unfortunately, this particular family was not further propagated, so that direct proof of its nature was not obtained. Indeed, it would seem that the unusual ratio which it shows must have been entirely overlooked, since the summary of the results with this strain (p. 307) commences with the words 'Thus every attempt to breed out the doubles proved unsuccessful', a conclusion which must be regarded as most unfortunate in view of the probability that doubleness would have been promptly 'bred out' had the first family which showed a decided deficiency of doubles been used for the purpose. That Winge is undoubtedly right in

regarding the usual excess of doubles as wholly due to differential viability, and this particular family as exceptional will be made clear in the following section.

## II. *A Graphical Method of Examining Frequency Ratios.*

The fact, which for many years misled geneticists with regard to the problem of double stocks, was that in the ever-sporting strains, the



○ Circles show tendency of percentage to approach 50 per cent. as families are made larger, i.e. as conditions affecting viability are improved. + shows aberrant value obtained by including the exceptional family.

Diagram showing proportion of singles according to the size of family.

numbers of single and double plants surviving to be classified, were not equal. A significant and fairly regular excess of double plants was constantly observed. Such departure from simple ratios may be due to unequal viability, or equally it may be suspected that a more complex genetic hypothesis is required. A simple graphical device, which, with appropriate data, will readily indicate, on internal evidence, that the whole of the discrepancy must be ascribed to unequal viability, may therefore be of use in other cases. Using the group of 87 Glabrous-red families, which were published by Miss E. R. Saunders in 1911, it will be seen that had any such device been applied to examine the data at this date, no

reasonable doubt would have remained that the ratio of singles to doubles was genetically a 1 : 1 ratio, in spite of the persistent and significant excess of doubles observed in most families.

The method consists in plotting the percentage of singles in families of different sizes. The boundaries between the successive size-classes are chosen to be in harmonic progression, with the upper boundary of the highest class at infinity. The successive boundaries are thus found by dividing some chosen number by 0, 2, 4, 6, . . . Since it is desirable, to avoid ambiguities, that these boundaries shall not be whole numbers, it is as well to choose, as the fundamental number, upon which the sequence is to be based, one that is odd. We require in addition that it shall not be so much as twice as great as the largest family, which in this case has 249 members. Further, since we shall use the same diagram to exhibit the aberrant character of a particular family of 31 members, we can make sure that this family shall be centrally placed in the group in which it falls by choosing a multiple of 31. We shall choose  $31 \times 13$ , or 403, and use the group limits found by dividing 403 by 2, 4, 6, 8, 10, 12, and 14; the values of which to one decimal place are given at the top of the diagram. All families of more than 28 members can be placed in one of these seven classes; the total numbers of singles and doubles obtained from each class can be enumerated, and the percentage of single plants plotted, as in the diagram. The horizontal line on the left of the diagram indicates the percentage of singles in families of less than 29 individuals, which have not been further subdivided. The Table below shows the totals in the different classes:

*Summary of 87 Glabrous-red Families; Data Plotted in Diagram.*

Size of family.	Single.	Double.	Total.	Percentage Single.
Over 201	239	250	489	48.88
101 to 201	433	503	936	46.26
68 to 100	261	321	582	44.85
51 to 67	180	235	415	43.37
41 to 50	102	157	259	39.38
34 to 40	130	202	332	39.16
29 to 33 (all families)	(98)	(118)	(216)	(45.37)
29 to 33 (Omitting 1 family)	75	110	185	40.54
Less than 29	200	303	503	39.76

It will be seen that the first four classes, where the percentages are based on the counts of more than 400 plants, point unmistakably to 50 per cent. (the black spot on the diagram) as the limiting value to which the percentage tends as the causes of mortality are more and more thoroughly removed. It has happened in this material that size of family has provided a sufficiently good basis for estimating the favourableness of the conditions in which the plants were reared, and, by extrapolation, for



inferring the genetic ratio appropriate to ideal conditions. In other cases it might be necessary to use germination percentage rather than size of family as a basis for classification. The method merely brings to our notice such indications, whether they are only slight, or, as in this case, so strong as to be decisive, as the data happen to contain. That the internal evidence should be so decisive, is a remarkable tribute to the intrinsic excellence of the data.

We may now consider the exceptional family with 23 single against 8 double plants. A glance at the diagram shows that the expectation for families of this size is about 40 per cent. singles, or roughly, 12 single plants to 19 double. The existence of families with a large excess of doubles (e.g. 9 singles to 23 doubles in family 6) would therefore be not intrinsically improbable, and their occurrence would be no reason for disregarding the family with an exceptional excess of singles. If any doubt remained on this point, or if this particular family had been overlooked, the percentage of singles obtained for this size-class, and represented by a cross on the diagram, would have shown that the inclusion of this family had been sufficient to disturb the average of its class, to an extent which the regularity of the other points shows at once to be inadmissible.

The same diagrammatic examination of the frequency ratio, would therefore, in this case, have provided decisive evidence that the discrepancy from a 1 : 1 ratio was due to differences of viability only ; and, at the same time have drawn attention to the really exceptional character of family 86.

### III. *Modification of Linkage Intensity.*

In discussing the origin of the ever-sporting type Winge (p. 131) naturally suggests that the first step was the occurrence of the mutation for double flower, which can at first only have been perpetuated by the continual selection by the growers. Selecting singles from families, or seed batches, which threw the highest proportion of doubles they would have immediately seized upon and brought into general prevalence the ever-sporting type of single, as soon as the pollen lethal had occurred in the single-bearing chromosome of a heterozygote. Winge quotes Miss Saunder's conclusion that the ever-sporting combination must have come into existence before the end of the seventeenth century, possibly not long after the double-flowered type was first known, but he does not call attention to the interesting process of selection which, according to his theory, must have taken place during the 200 or 250 years, since the pollen lethal was introduced. During this period the ever-sporting types of stock must have retained, generation after generation, only the progeny of non-cross-over gametes, for the cross-overs, as we have seen, would rapidly degenerate into singleness, and, though they might occasionally form new single

varieties, the effect is the same as though they were necessarily discarded, for their germ plasm will not again be introduced into the ever-sporting strain. Within such strains, therefore, selection must constantly have favoured closer linkage, and this explains what would otherwise have to be regarded as a somewhat remarkable coincidence, namely, that the lethal needed to balance the doubleness should have occurred exactly where the gardener wanted it, or at least within about 1 cross-over unit of the gene for doubleness. Recognizing that cross-overs have been rigorously eliminated we might suppose, on the other hand, that the cross-over percentage originally was as high as 10, perhaps, or 20 per cent. and that it has been lowered progressively by the selective elimination of those strains in which recombination took place most freely.

The conclusion that the extremely close linkage observed is due to the recent action of relatively intense selection is supported by three further facts:

(i) Although two of the races used by Miss Saunders, the Glabrous-red and the Sulphur-white, were composed entirely of ever-sporting individuals, yet the Glabrous-white and Cream plants were mixed in type, some breeding true to singleness, others throwing three singles to one double, and others again throwing an excess of doubles like other ever-sporting plants. On Winge's hypothesis such a mixture would inevitably arise sooner or later in the propagation of pure lines of seed, without any outside contamination, simply by the occasional occurrence of cross-overs, and the elimination of doubleness in their descendants. The frequency of such impure batches of seed, suggests that in most strains the frequency of recombination is higher than in the reliable ever-sporting strain tested by Winge.

(ii) An even more striking example of the continued existence of occasional individuals with relatively high frequencies of recombination is afforded by plant K of Miss Saunders's Cream line. Progenies were grown from 49 single-flowered offspring of this plant and of these, though certainly the majority were true to the ever-sporting type, at least five showed good 3:1 segregations, and five more gave only singles. Winge says 'Miss Saunders is herself at a loss for an explanation of the phenomenon, and my theory does not give any satisfactory explanation either. For it would imply a rather frequent crossing-over, and this is contrary to the findings in other experiments. I am rather inclined to think that we are here dealing with an experimental error, but, of course, it is not very satisfactory to try to explain the results of other investigators by ascribing them to some slip in the technique'.

Now if the close linkage observed in some reliable ever-sporting strains is due to the selection of the last 200 years it is by no means improbable that other less reliable strains have persisted in which the

frequency of crossing-over is considerably higher. In such strains we should expect most frequently to come across such an apparent 'mixture of seed' as is noticed by Miss Saunders, and also such plants as K of the Cream strain, which, while still of the balanced-lethal constitution, have so high a rate of crossing-over, that, if they are largely used as parents, their offspring will rapidly show signs of such mixture. There is thus no reason to suspect a slip in technique and, indeed, the plant may be cited as a remarkable confirmation of one of the most interesting consequences of Winge's theory.

(iii) The effect of selection in favour of close linkage between two loci must be to diminish principally the crossing-over between them; but also, presumably in less degree, in the two adjacent segments of the same chromosome. Its observational effect will therefore be to shorten greatly in the intervening segment, and to some extent elsewhere, the map length of the chromosome in question, and so to increase the probability of other close linkages found in the same chromosome. It is therefore relevant to the view that the close linkage between the factor for doubleness and the pollen lethal found with it, in ever-sporting strains, has been produced by the selection inherent in the propagation of these strains, that these two factors are both closely linked with the factor for yellow plastids.

#### SUMMARY.

1. An outline of Winge's theory of doubleness in stocks is given, and of its implications.
2. A simple method of diagrammatic representation applied to Miss Saunders's data of 1911, shows both that the observed excess of doubles is due solely to their greater viability, and that one family there reported was exceptional in giving only one quarter doubles, as should the progeny of a plant freed from the pollen lethal.
3. The close linkage between the pollen lethal and the factor for doubleness is due to selection acting automatically in the propagation of the ever-sporting lines, which has thus built up the ever-sporting character.



# Saltation Induced by X-rays in Seven Species of *Chaetomium*.

BY

HUGH DICKSON.

(From the Department of Botany, Egyptian University, Cairo.)

With Plates XXIV and XXV and twenty-seven Figures in the Text.

## A. INTRODUCTION.

IN a previous paper (4), a number of different fungi were X-rayed, and in three of them, *Phycomyces Blakesleeanus*, *Mucor genevensis*, and *Chaetomium cochliodes*, saltants were produced as a result of the treatment. The saltants appeared as variant sectors in colonies arising from subcultures obtained from the irradiated material (mycelium with or without reproductive organs), and also, in the case of *C. cochliodes* as saltant colonies each derived from a single irradiated ascospore. The frequency of saltation was very much higher, and the range of variant characters covered by the different saltants was very much wider in the case of *C. cochliodes* than in that of either of the other two fungi. In view of this it was thought worth while to extend the investigation to other species of the genus *Chaetomium*, and in this way to determine whether the ready response to X-ray treatment is confined to *C. cochliodes*, or whether it is characteristic of the genus as a whole. Six other species of *Chaetomium* were accordingly obtained, and these, together with *C. cochliodes*, formed the material for the present investigation.

Since in the paper referred to above no work was done on the growth characteristics of the saltants on different media, a number of experiments have now been carried out on the growth forms and rates of spread of several saltants and their parent strains. These have been grown on synthetic media in which the concentrations of the chief constituents have been varied, and dissimilarities in the response of the saltants and their parents to the different growth conditions recorded.

## B. MATERIALS AND METHODS OF EXPERIMENT.

*C. cochliodes* Pall., was isolated from an *Antirrhimum* stem and has now been in culture for upwards of three years. Miss W. M. Page of

Birkbeck College kindly provided cultures of *C. botrychodes* Zopf, *C. caprinum* Bainier, *C. globosum* Kze., and *C. murorum* Cda., and these, together with the two species *C. Fieberi* Cda., var. *rufipilum* (Grove) Sacc., and *C. elatum*<sup>1</sup> Kze., have now been in culture for periods ranging from two to five years.

Malt-agar (1.5 per cent. malt, 1.5 per cent. agar) was used in all cases as the culture medium, unless otherwise stated. In preparing the material for irradiation each species was subcultured by single hyphal-tip inocula, and each inoculum was placed in a separate Petri dish which was then incubated at 25° C. After four days the peripheral half-centimetre of each colony was removed, cut into pieces of convenient length, and placed in another Petri dish. The peripheral strips of all the species were placed in the same dish, the material of each species being kept separate from that of the others (since the material consisted entirely of mycelium bearing no spores of any kind, the risk of admixture of the species was negligible and no case was found of its having occurred). The Petri dish was then exposed to X-radiation, the glass lid being replaced by a celluloid screen during exposure. The X-ray tube was of the Coolidge type and was worked at a potential of 64 kilovolts and a tube current of 5 milliamps. The distance of the Petri dish from the target was 20 cm., and the time of exposure 200 minutes. Immediately following irradiation the material of the different species was cut into pieces of equal size, and each piece was used as an inoculum. Ten inocula of the same species were placed in each of twenty dishes, thus giving 200 colonies per species and 1,400 in all. The dishes containing the inocula were incubated for thirteen days at 25° C, when the number of saltants in each colony was counted.

On malt-agar all the species developed much the same amount of mycelium, but the production of perithecia varied greatly from one species to another. By taking inocula from the growing edge of the colony where perithecia had not been produced, and by using inocula of as nearly the same size as possible it was considered that the amounts of each fungus so transferred would be sufficiently similar to enable a reliable comparison to be made of the relative numbers of saltants induced in the various species by irradiation.

### C. THE FREQUENCY OF INDUCED SALTATION IN THE DIFFERENT SPECIES.

The following table gives the mean number of saltant sectors per ten subcultures for each of the seven species, arranged in ascending order:—

<sup>1</sup> I have much pleasure in thanking Miss F. L. Stephens of the British Museum (Natural History), who very kindly identified the majority of the species.

	Mean number of saltants per ten subcultures.	S.E. %
<i>C. Globosum</i> . . . . .	0.9	± 18.9
<i>C. Fieberi</i> , var. <i>rufipilum</i> . . . . .	2.3	± 16.8
<i>C. botrychodes</i> . . . . .	2.8	± 9.8
<i>C. cochliodes</i> . . . . .	3.0	± 13.0
<i>C. elatum</i> . . . . .	12.6	± 6.2
<i>C. murorum</i> . . . . .	14.5	± 6.4
<i>C. caprinum</i> . . . . .	25.5	± 4.2

From the table it is seen that *C. cochliodes* occupies an intermediate position as regards frequency of saltation, three species saltating more frequently and three less frequently. It is noticeable, however, that the seven species fall into two distinct groups which have very different frequencies, the first four species in the table saltating much more rarely than the remaining three.

A large number of unirradiated cultures of each of the parent strains has been grown in order to ascertain their variability or otherwise, and it was found that while *C. Fieberi* var. *rufipilum* and *C. murorum* do saltate very occasionally, the other five species are quite stable, no case of saltation having been observed. Since of the two species which show variability under normal growth conditions one saltates frequently as a result of irradiation, and the other infrequently, there does not appear to be any correlation between the variability of the fungus unirradiated, and the frequency of saltation following irradiation. This is in agreement with the results previously obtained (4) where it was shown that, whereas the three fungi which did saltate on irradiation were very stable under ordinary conditions, two species of *Fusarium* which normally saltate fairly frequently were, in this respect, completely unaffected by exposure to X-rays.

Chivers (3) regards *C. Fieberi* as synonymous with *C. globosum*, and in view of this it is of interest to note that, although *C. Fieberi* var. *rufipilum* has a frequency of saltation much closer to that of *C. botrychodes* or *C. cochliodes* than to that of *C. globosum*, *C. Fieberi* var. *rufipilum* and *C. globosum* are adjacent to one another in the table.

In all cases saltants appeared in the form of simple sectors and in no case was a variant obtained from any part of a colony other than from such sectors. Cases such as those recorded by Brown (2), and by Horne and Das Gupta (5) in which saltant strains have arisen from inocula taken from the older parts of apparently homogeneous colonies have not occurred. In the majority of cases the sectors have arisen in the centre of the colony (Pl. XXV, Fig. 7) though not infrequently the apex of the sector is some distance away from the centre (Pl. XXIV, Fig. 6). In most cases also the saltant has a growth rate approximately the same as that of the parent, so that neither strain increases at the expense of the other. Examples have

been obtained, however, in which the saltant has a greater growth rate than the parent, with the result that the lines of junction of the parent and saltant are no longer straight (as is the case when the growth rates are equal), the line of junction on one side of the saltant curving away from that on the other side (Pl. XXIV, Fig. 5). In cases where the growth rate of each of the two strains is constant, and there is no effect on the growth rate of either strain due to the presence of the other, it can be shown that the line of junction between the two strains forms part of an equiangular spiral. In a few cases such as that shown in Pl. XXIV, Fig. 6, the saltant failed to expand as the colony increased in diameter, and was eventually cut off by the parent strain (see also Dickson (4), Pl. XIII, Fig. 6). Such cases are probably due to a slower growth rate on the part of the saltant, and this may be combined with differences in the staling characteristics of the two strains. That such simple explanations as these are not always correct, however, has been found by the writer in the case of a saltant of *Coprinus* sp. The saltant sector in this case diverged rapidly, but it was found on culturing the saltant that it grew less quickly than the parent strain, and that neither strain staled when grown either alone or in proximity to the other. Further investigation of this is at present in progress.

#### D. CHARACTERISTICS OF THE SALTANTS.

After counting the number of sectors found in the different colonies subcultures were made of the various variant types exhibited by each species. These were left to grow, and if on examination sectoring was still observed fresh subcultures were made. This was repeated until a non-sectoring colony was obtained, when a single hyphal-tip subculture was made which formed the starting point of stock cultures of that particular saltant. In this way ten saltant strains of each species were obtained; these were chosen from the original sectors so as to constitute as representative a series as possible of the different saltant types.

In making an examination of the characteristics of the different saltants a number of different media were used, of which malt-agar proved the most suitable medium for colour production and the formation of aerial mycelium. Perithecia also were readily produced in most cases on this medium, but owing to the aerial mycelium making examination of the perithecia difficult horse or goat dung was used for this purpose whenever possible. On either of the last two media perithecia were in general freely produced (exceptions being *C. Fieberi* var. *rufipilum* and *C. elatum*, in which few perithecia were produced on horse dung and none on goat dung, though they were very numerous on malt in both cases), and except in the case of one or two saltants, e.g. C3, scarcely any aerial mycelium was formed. Some saltants did not produce perithecia on any of the above media, and such cases were



then cultured on prune, raisin, potato extract, and Brown's synthetic medium before being recorded as sterile.

In the following description it was thought that a general account of the variations exhibited by the saltants of each species would be of wider application than would a detailed account of each saltant, accordingly, except in specific cases, only a comparative description of the various variant characteristics has been given. A description of the *C. cochliodes* saltants is included for the sake of completeness, though a detailed account has been given elsewhere (4). The colours quoted below are those of Ridgway's colour standards.

*Chaetomium botrychodes*.

In this species little coloration of the substratum was produced, and the range of colour exhibited by the saltants was also limited. The colour of the parent strain was pinard yellow, and the saltants showed a range varying from colourless through lemon and baryta yellow to empire yellow. The number of perithecia produced showed considerable variation, one saltant being completely sterile, whereas a second formed a colony whose surface was densely crowded with perithecia, and intermediate stages between these two were also found. The sizes of the perithecia of the different saltants showed considerable variation, none of the saltants produced perithecia appreciably larger than the parent (Text-fig. 9), but various degrees in reduction of size were found (e.g. Text-fig. 10). The perithecial hairs, which are of primary importance in determining the species, likewise showed some variation. In one saltant (Text-fig. 11) the hairs were relatively straight, in another (Text-fig. 12), while they were coiled, they were far from having the regular close spiral characteristic of the parent strain. The strain shown in Text-fig. 10 produced no asci.

*Chaetomium caprinum*.

As in the previous strain variation in the colour of the substratum was small, and the chief differences between the saltants and the parent lay in the perithecia. Text-fig. 13 shows a perithecium of the parent strain, the saltant shown in Text-fig. 14 has a smaller development of the apical hairs, and that in Text-fig. 15 produces hairs less regularly coiled than in the parent. In the last case also, though ascospores are plentifully produced, they are not extruded as is normally the case.

*Chaetomium cochliodes*.

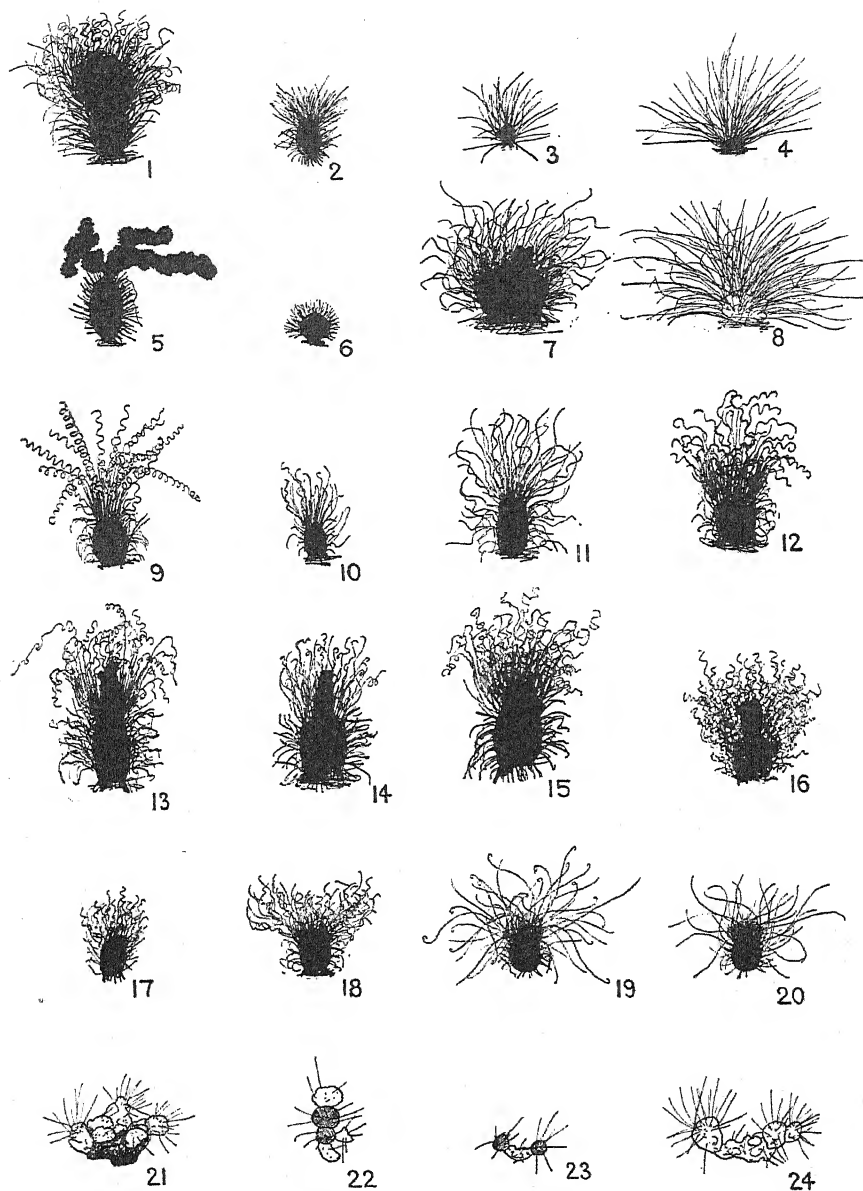
The number of saltants of this species which has been examined was much greater, and the extent of the variation of the different saltants from the parent was also greater than was the case with any of the other species. It is probable that this larger variation is due to the greater number of

saltants examined rather than to any innate capacity of this species to produce a wider range of saltant types. As was the case in the majority of the species, the most marked variability was shown by the perithecia. Text-fig. 1 shows a perithecium of the parent strain, and Text-figs. 2-8 are examples of the type of variation found. As can be seen from the figures all degrees of reduction in size and considerable variation in shape of the perithecia were obtained. Differences in the degree of coiling of the hairs, and also in their length were found. In Text-fig. 5 the hairs are short, quite straight, and are remarkable by reason of the bluntness of their tips which in the other saltants and in the parent taper insensibly to fine points. In this strain also the spores were produced so freely that on being extruded they gave a dark brown or black appearance to the mass of perithecia in a colony. The body of the perithecium shown in Fig. 8 was not coloured a dark olive-green as was normally the case, but remained a yellowish white. Perithecia of a Mars yellow colour darkening as they get older to antique brown have been produced twice (Pl. XXIV, Fig. 2). Coloration of the substratum, which in the parent was very slight (Pl. XXIV, Fig. 1), was obtained in a number of cases. In the majority of these the colour was brown, but on three occasions a reddish hue was obtained, and one of the latter (SS9),<sup>1</sup> is shown in Pl. XXIV, Fig. 3. In this case the colour is a peach red, which, as the culture gets older, darkens to nopal or Brazil red. Some saltants produced more and some fewer perithecia than the parent, but in one case only was the distribution of the perithecia on the surface of the colony altered. In this variant the perithecia, instead of being evenly distributed as in the case of the parent and of the other saltants, were arranged in groups of two or three at the centre of the colony, though towards the edge they were produced singly.

### *Chaetomium elatum.*

Neither the parent strain nor any of the saltants produced perithecia on dung-agar. On malt-agar the parent strain and one of the saltants fruited readily, though the other saltants continued sterile. The number of perithecia produced by the fertile saltant was about equal to that of the parent; they were, however, smaller, and the hairs shorter with very little branching. In the parent very little colour was produced in the substratum, but in two of the saltants a raw sienna colour was obtained. Most of the saltants had a greater amount of aerial mycelium than the parent strain, the colour of the mycelium in the parent and in eight of the saltants was a dark olive-buff, while in the remaining two saltants it was white and pale pinkish buff, respectively.

<sup>1</sup> The letters SS signify a saltant derived from an irradiated spore as against one arising from irradiated mycelium with or without perithecia.



TEXT-FIGS. 1-24. For explanation see text.

*Chaetomium Fieberi* var. *rufipilum*.

In all the saltants except two, perithecia were absent. In the case of both the fertile strains perithecia were few in number and small in size, the

hairs being longer than in the parent and straight. Slight variations in the colour of the aerial mycelium and in the substratum were present, but were not well marked. One of the saltants staled strongly on malt-agar, and formed a dense web of mycelium.

*Chaetomium globosum.*

Of the various species used in these experiments *C. globosum* most closely resembles *C. cochliodes* both in perithecial characters and in its appearance in culture. It produced a saltant G1 which colours the substratum orange chrome (Pl. XXV, Fig. 9); this colour is only slightly more orange than that of the saltant of *C. cochliodes* shown in Pl. XXIV, Fig. 3. This saltant (Text-fig. 17) has smaller perithecia than the parent (Text-fig. 16), but in the amount of coiling of the hairs they are very similar to one another. A second saltant (G2) produced a very similar colour in the substratum (Pl. XXV, Fig. 10), and at the same time showed marked staling characteristics on malt-agar. The perithecia differed from the parent in that the terminal hairs spread out in the form of a hollow cone (Text-fig. 18), thus leaving the ostiole freely exposed. The remaining saltants showed slight differences in the amount of aerial mycelium and in the number and size of the perithecia.

*Chaetomium murorum.*

Four of the ten saltants showed complete sterility on both malt- and dung-agar. The strain M2 produced only small perithecia with relatively long hairs. M6 (Text-fig. 20), had perithecia similar to the parent (Text-fig. 19), except that they were somewhat smaller, and the terminal hairs did not show circinate curvature of the tips. This absence of curvature is a characteristic of young perithecia of the parent strain, and appears to have become an adult feature in the variant. Three saltants had normal perithecia. The remaining saltant, M3, produced a type of modification which has not been seen in any other variant. The bases of the perithecia branch and the apices of the branches are in some cases surrounded by straight and white hairs, while in others they are more or less glabrous. Some of the groups are larger than those shown in Text-figs. 21-4, and may have as many as ten or twelve ultimate branches. The majority of the perithecia, instead of being dark brown or black, as is normally the case, are white (Fig. 24), though occasionally (Figs. 21-3), parts of the perithecia are dark and the rest white. All the perithecia of M3 are sterile.

*C. murorum* differed from all the other species in the readiness with which the colour present in both the mycelium and substratum was altered, seven of the ten saltant strains showing variation in this respect. The parent strain was ecru-olive in colour (Pl. XXIV, Fig. 4), M1 (Pl. XXIV, Fig. 5), and M2 were colourless, and M3 (Pl. XXV, Fig. 6), M5 and M7 were yellowish

oil green, citrine, and sulphine yellow respectively. The mycelium of M6 (Pl. XXV, Fig. 7), in young colonies coloured the medium a redder hue than did the young mycelium of an older colony. In old cultures the central portion became yellower than it was originally, and assumed a cinnamon rufous colour. It always remained redder, however, than the peripheral area. M8 was very similar to M6 except for the absence of the reddish hue in the centre of the colony.

The amount of aerial mycelium was about equal in all the saltants and the parent, with the exception of M<sub>1</sub>, M<sub>2</sub>, and M<sub>3</sub>, where it was considerably more abundant, and M6 and M8, where it was thinner.

The uneven growth of the parent colony over the surface of the medium (Pl. XXIV, Fig. 4), which is probably a result of staling in a mild form, while retained in most saltants was absent in others, for example, M6 (Pl. XXV, Fig. 7).

In four of the seven species, namely, *C. cochliodes*, *C. elatum*, *C. Fieberti* var. *rufipilum*, and *C. murorum*, strains producing dense white aerial mycelium have been obtained. These have in all cases, with the exception of M<sub>2</sub>, been completely sterile. They show slight differences from one another in the glossiness and in the amount of the aerial mycelium formed, and also to some extent in the evenness or otherwise with which it is produced. It is probable that a variation in the cultural conditions would show other differences between the strains, as it has been found in the case of C<sub>3</sub>, a saltant of *C. cochliodes*, that on growing it on the synthetic medium devised by Brown (1) with an increased amount of glucose, a pale yellow colour was given to the mycelium.

The majority of the saltants have proved to be stable in culture, but occasionally variants of the saltants have appeared as sectors. This has been most noticeable in the case of the saltants of *C. murorum*, in all of which saltation occurs occasionally (cf. Pl. XXIV, Figs. 5, 6, and Pl. XXV, Fig. 7). The saltant of *C. globosum*, G<sub>2</sub>, which colours the medium orange-chrome, has on one occasion thrown a saltant which produces practically no coloration of the substratum, less in fact than the parent strain. This saltant stales on malt, but less strongly than is the case with G<sub>2</sub>.

While the number of saltants grown does not justify an exact comparison being made between the variant characteristics of the saltants of the various species, certain broad inferences are possible. In general it can be said that each species has produced saltants whose variant characters affect all parts of the plant, and this applies not only to morphological characters such as the structure of the perithecia and the type of vegetative growth, but also to such physiological changes as differences in the growth rate, and the colour produced in the substratum. Certain differences between the species are seen in the readiness with which the various characters may be altered. Thus *C. murorum* is exceptional in the

number of its saltants which show marked colour changes. Colour is also more readily altered in *C. globosum* and, to a less extent, in *C. cochliodes* than it is in the other species. *C. elatum* and *C. Fieberti* var. *rufipilum* are notable for the number of sterile saltants they have produced, and *C. elatum* and *C. murorum* for the number of saltants in which the amount of aerial mycelium has been increased over that of the parent strains.

Despite the large changes produced in the perithecial characteristics of the different species no case has been found of a saltant having characters more like those of another species than of that from which it was derived, the general tendency has been rather in the direction of producing saltants whose perithecia are of a common type in that they are small, have straight terminal hairs, and are relatively sterile.

#### E. THE EFFECT OF VARIATIONS IN THE CULTURE MEDIUM ON CERTAIN SALTANTS AND THEIR PARENT STRAINS.

The synthetic medium<sup>1</sup> devised by Brown (1) has been used as the standard medium. This has been varied in respect of the three main constituents, namely, asparagin, glucose, and phosphate, and the effect of such variations on the growth rate and form of each of the strains determined.

The following six strains have been compared in this way, *C. cochliodes* parent (Cp), and its two saltants SS9 and C3, and *C. murorum* parent (Mp), and the saltants M3 and M6. Five plates of each strain on each of the various media have been grown throughout, and in cases where the growth rates showed wide variations over a narrow range of media the experiment was repeated for the particular concentrations concerned.

#### I. THE PHOSPHATE CONTENT.

Four different concentrations of  $K_3PO_4$  were used, namely 0.0, 2.0, 4.0, and 8.0 gm. per litre, and the standard medium was also altered by the addition of 0.4 per cent. potato starch and by altering the amount of glucose from 0.2 to 0.4 per cent.

No staling, as indicated by a falling off in the growth rate, was found in any of the six strains at any of the four concentrations of phosphate. The curves showing the relation between diameter of colony and time were very approximately straight lines from the time measurements were begun on the second day till they were stopped on the eighth, in all cases. At the various concentrations of phosphate differences in the growth rates of the several strains were observed. In general the growth rates increased with increasing concentration, and this tendency was more marked in the case of the three *C. murorum* strains than in the strains of *C. cochliodes*. The

<sup>1</sup> Glucose 2.0 gm., asparagin 2.0 gm.,  $K_3PO_4$  1.25 gm.,  $MgSO_4$  0.75 gm., agar 25 gm., water 1,000 c.c.

three *C. cochliodes*' strains differed from those of *C. murorum* in that the growth rate was less at 0.4 per cent. phosphate than at 0.2 per cent., this drop being more marked in the case of C<sub>3</sub> than in either Cp or SS<sub>9</sub>.

Little difference was produced in the amount of aerial mycelium formed in the case of Cp and SS<sub>9</sub> as the amount of phosphate was increased; this was also the case with the three strains of *C. murorum* which, however, only produced a very scant aerial mycelium at any concentration. In the case of C<sub>3</sub> the aerial mycelium was unevenly produced on the surface of the colony when no phosphate was present, but with increasing amounts it became quite evenly distributed. Perithecia were only produced in the case of Cp and SS<sub>9</sub>. Increasing phosphate yielded increased numbers of perithecia with Cp, but with SS<sub>9</sub> the numbers remained almost constant while the size decreased as the concentration rose. Some coloration in the submerged mycelium was observed in Mp and M<sub>3</sub>, and this became less as the concentration of the phosphate increased. In M<sub>6</sub> colour was only produced in the vicinity of colonies of *Penicillium*. SS<sub>9</sub> failed to produce a reddish coloration at any concentration.

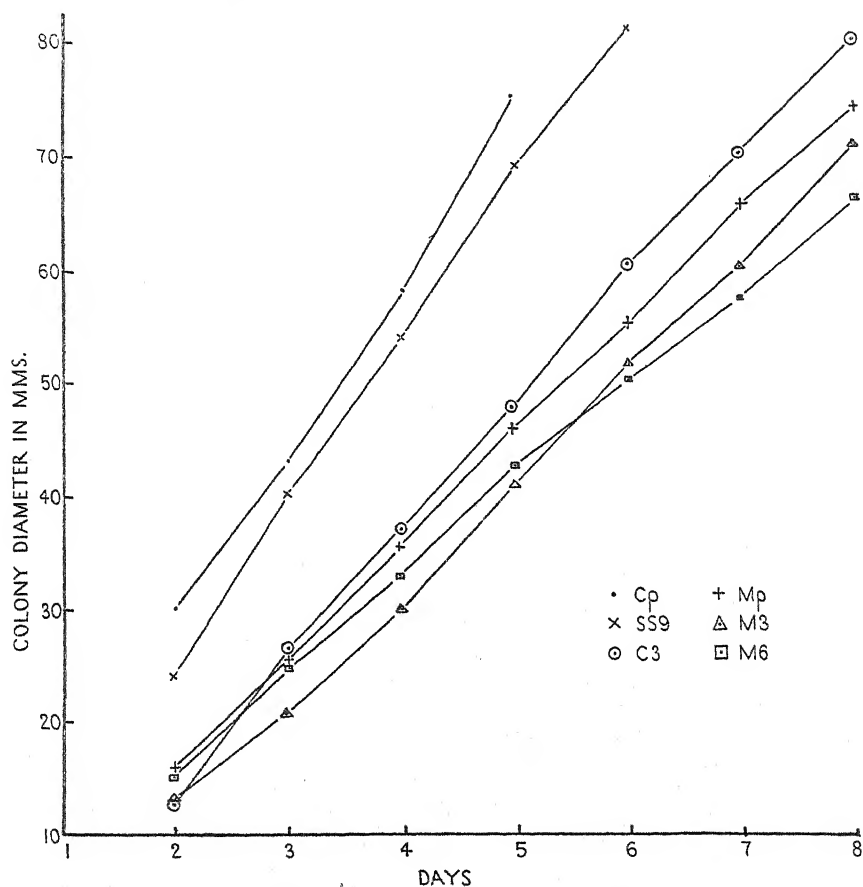
## II. THE GLUCOSE CONTENT.

In determining the effect of the glucose constituent the standard medium was made up without the addition of starch. The following concentrations of glucose were used:—0.4, 0.8, 1.6, 2.4, 3.2, 4.0, 5.2, and 5.6 per cent.

The results of the measurements of the growth rate are shown in Text-figs. 25 and 26. Text-fig. 25 shows the diameters of the different strains from the second to the eighth day on the medium containing 4.0 per cent. glucose, and is typical of the results obtained at each of the other concentrations. It will be seen that in no case is there any falling off in the growth rate, so that on the eight media under consideration no staling occurs in any of the strains. The growth rate remains practically constant from the second day when measurements were begun till the colonies had reached a diameter of 65–80 mm. when measurements were discontinued, except in the case of M<sub>3</sub> where there is a tendency for the growth rate to go on increasing up to the third or fourth day. This increase in the rate of M<sub>3</sub> has been observed at all the concentrations of glucose, and is better seen at other concentrations than in the one illustrated; in no case, however, is it strongly developed.

In Text-fig. 26 the mean increase in diameter per twenty-four hours growth for each strain is plotted against the different concentrations of glucose. The mean increase was taken as the average diametric advance per day over the whole period of growth in each case. On comparing the growth rates of the different saltants with their parent strains it can be seen that there are clearly defined differences. With increasing glucose

concentration the growth rate of Cp rises steadily at first and then falls abruptly at a concentration of 3.2 per cent. Above this point it again increases, at first rapidly and then more slowly.



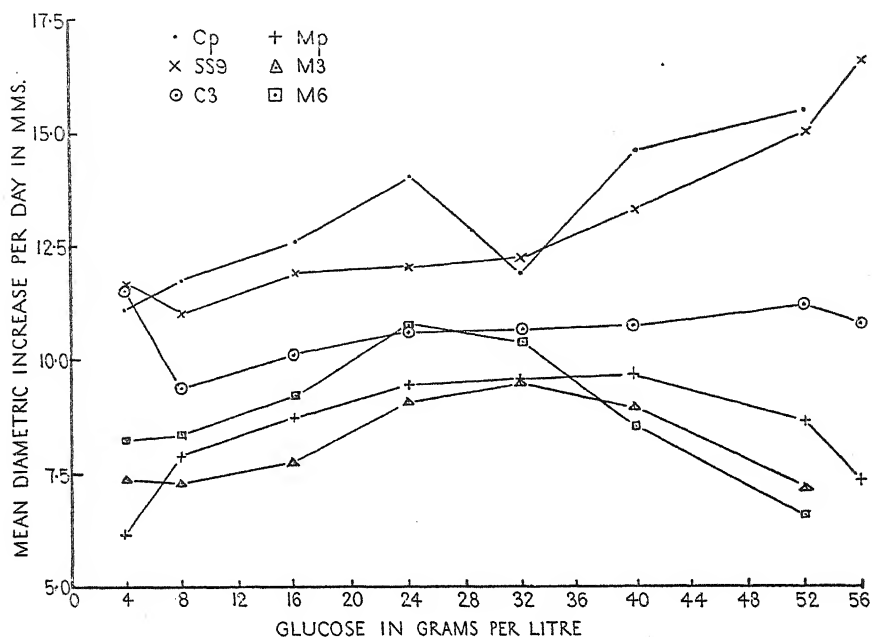
TEXT-FIG. 25. For explanation see text.

In contrast to this SS9 has a relatively smooth curve. Following an initial fall in the growth rate between 0.4 and 1.6 per cent. the rate increases with increasing concentration, the gradient gradually becoming steeper up to the maximum concentration of 5.6 per cent. C3, while showing an initial fall in the growth rate as is the case with SS9, and at approximately the same concentration, exhibits a slow but practically constant increase in the growth rate up to 5.2 per cent., when the rate falls off somewhat sharply.

The main differences then between the growth rates of these three strains are as follows:—Cp on the average grows more rapidly than SS9, and SS9 more rapidly than C3. A sudden falling off in the growth rate



occurs between 0.4 and 1.6 per cent. in the case of SS9 and C<sub>3</sub>, and between 2.4 and 4.0 per cent. with Cp. In general the growth rate of each of the strains increases with increasing glucose. The gradient of the curve of Cp



TEXT-FIG. 26. For explanation see text.

is at first steady, but later falls off; in SS9 it gradually gets greater with increasing concentration, and with C<sub>3</sub> it is practically constant up to a concentration of 5.2 per cent., when the growth rate suddenly decreases.

The curves of the growth rates of *C. murorum* and its two saltants show smaller differences from one another than do those of *C. cochliodes*, and unlike them are devoid of any sudden changes in direction. All the curves show at first an increase in the growth rates and later a decrease. In the case of M<sub>3</sub> and M<sub>6</sub> the rate of this increase goes up, whereas in Mp it falls off as the concentration becomes greater. The concentration at which the growth rate is a maximum differs slightly in each of the strains. In the case of M<sub>6</sub> it occurs at 2.4 per cent., in M<sub>3</sub> at 3.2 per cent., and in Mp, where the maximum is much less sharp than in either of the saltants, at 4.0 per cent.

The changes produced in the appearance of the colonies by variations in the percentage of glucose were small. In Cp and SS9 there was a very slight increase in the amount of aerial mycelium as the concentration increased, and this was accompanied by a reduction in the number of perithecia. No reddish coloration occurred in SS9 at any concentration,

C<sub>3</sub> showed little increase in the amount of aerial mycelium. At the lowest concentration (0.4 per cent.) the mycelium was coloured a pale lemon yellow, the colour being strongest round the edge of the colony, with concentrations between 0.4 and 2.4 per cent. the colour decreased, and above 2.4 per cent. the mycelium was pure white. The effects of alteration in the percentage of glucose on Mp, M<sub>3</sub>, and M<sub>6</sub> were small, the amount of aerial mycelium being little affected and changes in coloration slight.

### III. THE ASPARAGIN CONTENT.

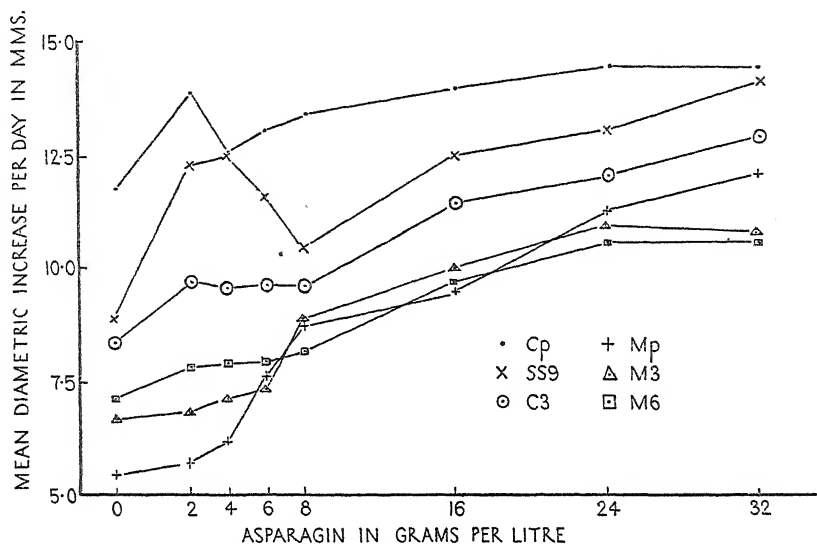
The growth rates on the standard medium to which was added amounts of asparagin varying from 0.0 to 3.2 per cent., showed that five of the six strains were definitely nonstaling, and the sixth strain, C<sub>3</sub>, only showed slight staling, as indicated by a falling off in the growth rate, when the asparagin content reached 2.4 per cent. With this exception each of the strains at all concentrations showed a remarkably constant growth rate between the times when measurements were begun, namely the second day after inoculation, and when they were stopped on the eighth day or on the colony approaching the edge of the Petri dish.

Text-fig. 27 shows the relationship between the concentration of asparagin and the mean daily increase in diameter of the colony over the whole period during which measurements were made. In the case of C<sub>3</sub> growing on the media containing 2.4 and 3.2 per cent. asparagin the mean rates were calculated on the measurements from the second day to the fifth at which time staling first became evident in each case.

*C. cochliodes* parent strain shows an initial rise in the growth rate on increasing the asparagin content from 0.0 to 0.2 per cent., the rate then falls to a minimum at 0.4 per cent. and after that it at first increases and then continues constant. SS<sub>9</sub> produces a curve similar to that of Cp, but the initial increase in the rate reaches its maximum, and the ensuing minimum occurs at concentrations of asparagin higher than in the case of Cp, i.e. at 0.4 and 0.8 per cent. respectively. Above 0.8 per cent. the gradient is steeper than with Cp and the growth rate does not become constant at the higher concentrations. In this latter respect it agrees very closely with the curve of C<sub>3</sub>, but this strain, though it shows an increase in the growth rate from 0.0 to 0.2 per cent., gives a practically constant growth rate from 0.2 to 0.8 per cent., in which respect it differs from both Cp and SS<sub>9</sub>. The three strains show considerable differences in their growth rates. At all concentrations Cp is the most rapid and C<sub>3</sub> the slowest, SS<sub>9</sub> occupying an intermediate position throughout.

The curves of the three strains of *C. murorum* do not differ from one another so greatly as do those of *C. cochliodes*. The parent strain (Mp), shows an initial increasingly rapid rise in the growth rate up to 0.8 per cent., followed by a more or less constant increase from 0.8 to 3.2 per cent.

M<sub>3</sub> behaves somewhat similarly to M<sub>p</sub>, but the initial increase in the rate is smaller, and between 2.4 and 3.2 per cent. the growth rate decreases. In M<sub>6</sub> the rate increases fairly regularly from 0.0 to 2.4 per cent. and then remains constant between 2.4 and 3.2 per cent.



TEXT-FIG. 27. For explanation see text.

In Cp the aerial mycelium was thin in the medium containing no asparagin; above this concentration it was more plentiful and about equally so at all concentrations. The colour of the colonies was greenish yellow throughout. With no asparagin added to the medium the perithecia were large and fairly numerous, at 0.2 per cent. the perithecia were smaller and about equally plentiful and were concentrated at the centre of the colony. Above 0.2 per cent. they remained small and centred, but decreased slightly in numbers as the asparagin increased. The perithecia in SS9 were affected similarly to those of Cp up to 0.2 per cent. asparagin, but above this concentration the numbers decreased rapidly, and from 0.8 to 3.2 per cent. none were produced. C<sub>3</sub> showed no colour in the aerial mycelium at any concentration, at 0.0 and 0.2 per cent. asparagin the mycelium was smaller in amount and unevenly produced, but above these concentrations it was plentiful and formed an even felt. Little difference was observed in the growth forms of the colonies of the three *C. murorum* strains at concentrations between 0.2 and 3.2 per cent. asparagin, the mycelium was extremely thin in all cases. The effect of concentrations below 0.2 per cent. will be dealt with when considering the glucose: asparagin ratio.

In old cultures all the strains showed an alkaline reaction with a pH

of about 8.5 in all the media which contained asparagin irrespective of the amount. The media to which no asparagin had been added were more acid, and the pH varied slightly from strain to strain. In Cp, for example, it was 7.5, in SS9 8.0, and Mp and M<sub>3</sub> gave a pH of 7.0. Ammonia was produced and was probably responsible to a major extent for the alkalinity of the medium.

#### IV. THE GLUCOSE-ASPARAGIN RATIO.

In order to ascertain the effects on growth form of variations in the carbohydrate: nitrogen ratio, a series of media was made up, each member of which contained the same amounts of phosphate and sulphate as the standard medium. Different percentages of glucose and asparagin were then added in various combinations as indicated in the following scheme:—

Asparagin.	Glucose.			
	0.1 %	0.2 %	0.5 %	1.0 %
0.00 per cent.	A	B	C	D
0.05    "	E	F	G	H
0.10    "	K	L	M	N
0.20    "	O	P	Q	R

Three saltants and one parent strain were grown on each of the media and gave the following results.

SS9. In the series A-D practically no aerial mycelium was formed; in all the other media it was well-developed and showed little difference in amount from one medium to another. An orange-vinaceous colour was produced in all the media containing no asparagin; this coloration was most marked at B, the intensity falling off with either an increase or a decrease of glucose. In the media containing 0.05 per cent. asparagin the colour increased from E, where it was very slight, to H. In this series the colour ranged from orange yellow at E to orange red at H. In the two series containing higher concentrations of asparagin the amount of red had decreased but the orange coloration was still present and increased in intensity as the glucose content rose.

The perithecia showed marked differences alike in size, distribution, and number at the various concentrations of both glucose and asparagin. Increasing amounts of either glucose or asparagin produced an increase in the size of the perithecia. This increase reached a maximum in both cases and was followed by a decrease. The largest sized perithecia occurred at A, E and F (about equal), M, and R. An increase in the glucose, and more especially in the asparagin content, up to a point caused a larger number of perithecia to be produced; above this value the numbers fell off. The concentrations of glucose and asparagin at which the greatest numbers occurred were the same as those at which the maximum sizes

were produced. At the highest concentrations of asparagin, and to a much smaller extent of glucose, the perithecia, instead of being evenly distributed over the surface of the medium, were mainly confined to a small area of about two cm. diameter at the centre of the colony.

The above observations may be summarized as follows:

Increasing glucose causes an increase in the intensity of the reddish colour of the substratum up to a point above which the coloration gradually diminishes. An increase in the amount of asparagin on the other hand produces a continuous decrease in the production of the red colouring matter, the reddish hue gradually being replaced by orange and finally by yellow. An increase in the concentration of either glucose or asparagin caused an initial increase in both the size and number of the perithecia; on still further increasing the percentage of either substance, however, the size and number again diminished. At the highest concentrations of both glucose and asparagin the production of perithecia was confined to the central portions of the colonies.

Mp. The amount of aerial mycelium was very small in any medium; it showed, however, a slight increase with increasing glucose, while the asparagin content had no apparent effect. Perithecia were produced in O and P. The media A-D were the only ones in which a blue colour was obtained, the intensity of which reached a maximum at C. In the media to which asparagin had been added, the colour was much weaker and changed from a yellow green to yellow brown as the concentration of asparagin increased. With increasing glucose the colour increased to a small extent, and at all concentrations of asparagin, excepting the A-D series, was greatest at the highest concentration, i.e. at H, N, and R.

M3. The effect of varying the concentrations of glucose and asparagin on the amount of aerial mycelium produced was much the same as with Mp. The colour changes were also similar to those of Mp, but the colour was more intense. Perithecia were produced at random throughout the series, but in no case were they plentiful.

M6. Aerial mycelium increased slightly with increasing glucose but appeared unaffected by the asparagin content, except in the series without any asparagin where it was completely absent. The reddish colour characteristic of this saltant increased with an increase in the glucose content up to a point and then got less. The maximum coloration in the series was reached at G, though M also was well coloured. The colour in M was a maximum for the 0.1 per cent. asparagin series and was about equal to that in H. Little reddening occurred in the O-R series, but the orange brown colour produced was greatest at R. The A-D series was entirely devoid of colour. Apart from the A-D series, in which there was no asparagin, the effect of increasing the concentration of asparagin was progressively to diminish the reddish colour. The effects on colour of both

glucose and asparagin then are very similar to those produced in the strain SS9, except that in that case colour occurred in the total absence of asparagin.

Considering the diversity in growth form between the three members of each of the species, which have been examined under different conditions of culture, they show a close similarity in growth rate over a wide range of media. In most cases the growth rate curves of the different strains of each species are very similar to one another, though they display minor characteristic differences in the positions of certain optima and minima with relation to the concentrations of the nutrient substances in the medium. This tendency to have a characteristic growth rate common to both the parent strain and its saltants has been observed by Brown (1) in the case of *Fusarium* species. Certain other growth responses obtained with *Chaetomium* are similar to those obtained by Brown with *Fusarium*. Thus in both cases the carbon: nitrogen ratio is the dominant factor in colour production, an increase in the asparagin content from the minimum being followed by an initial increase (probably due to the increased amount of possible growth) and then a rapid and continuous diminution in colour. An increase in the percentage of glucose caused intensification of colour up to a point, and this was followed by a decrease in intensity on the glucose content being still further increased. As regards sporulation, similar effects were obtained on increasing the phosphate content, in that with both the *Fusarium* species and the parent strain of *C. cochliodes* an increase in the percentage of phosphate was followed by an increase in spore production. No such effect was obtained with SS9, in which case the number of perithecia was practically unaffected, though at the higher concentrations their size was decreased. In *Fusarium* the effect of increasing the asparagin is to decrease sporulation, and high concentrations of glucose also cause its suppression. Somewhat different results from these were obtained in the case of the saltant SS9 in which as the asparagin or glucose content increased from the minimum the number and size of the perithecia were at first increased, but later the size diminished and the numbers were suppressed. In general the similarities in the effect on the growth form and colour production of variations in the culture media existing between these two widely separated genera are more striking than the differences they exhibit.

#### SUMMARY.

Mycelia of seven species of *Chaetomium* have been irradiated, and the relative numbers of saltants produced have been determined. The species were found to fall into two groups, the first of which contained four species and saltated relatively infrequently, while the second, with three species, produced saltants very readily.

A general account is given, together with figures of the saltant perithecia and photographs showing the colour changes, of the variant characteristics of the various species.

Two groups of strains, each consisting of a parent species and two saltants, have been grown on media in which the concentrations of the carbohydrate, nitrogen, and phosphate contents have been varied, and the resulting effect on growth form and rate of growth determined. The results have been compared with those obtained by Brown working with species of *Fusarium*, and certain similarities have been established in the reactions of the two genera.

The first part of this investigation was carried out at the John Innes Horticultural Institution, and I have great pleasure in recording my indebtedness to Sir Daniel Hall for his kindness in placing the facilities of the Institution at my disposal.

My sincere thanks are also due to Professor F. W. Oliver for much helpful criticism and advice.

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#### EXPLANATION OF PLATES XXIV AND XXV.

Illustrating Dr. H. Dickson's paper on 'Saltation Induced by X-rays in Seven Species of *Chaetomium*'.

The figures are reproduced from hand-coloured photographs taken by transmitted light, with the exception of Figs. 3 and 10 in which both transmitted and reflected light was used. The cultures were grown on malt-agar.

Figs. 1-3. *Chaetomium cochliodes*.

Fig. 1. The parent strain. The perithecia are dark olive green in colour, appearing black by transmitted light. The colour of the substratum is scarcely affected by the fungus.

Fig. 2. Saltant SS 8. The perithecia are smaller than in the parent and are Mars yellow in colour, deepening with age to antique brown.

Fig. 3. Saltant SS 9. Very similar to the parent, except for the strong coloration of the substratum.

Figs. 4-7. *C. murorum*.

Fig. 4. The parent strain. The mycelium is not very plentiful and is characterized by a peculiar flecked appearance, probably the result of a mild form of staling.

Fig. 5. Saltant M1. It produces a very abundant and practically pure white aerial mycelium. A sector can be seen in which the growth rate is greater than that of the parent, though otherwise the two are very similar.

Fig. 6. Saltant M3. It is similar to the parent strain in respect of the unevenness with which the mycelium is produced. It has, however, a much more abundant mycelium which is coloured a yellowish oil green. A narrow golden yellow sector can be seen at the edge of the colony.

#### PLATE XXV.

Fig. 7. Saltant M6. In contrast to Mp produces a very even mycelium which is thinner than in the case of the parent. The medium in old cultures, such as that illustrated, is coloured a somewhat redder hue at the centre of the colony than at the edge. A sector occupying about one third of the colony is seen; this is somewhat thinner than its parent but produces the same colour effects.

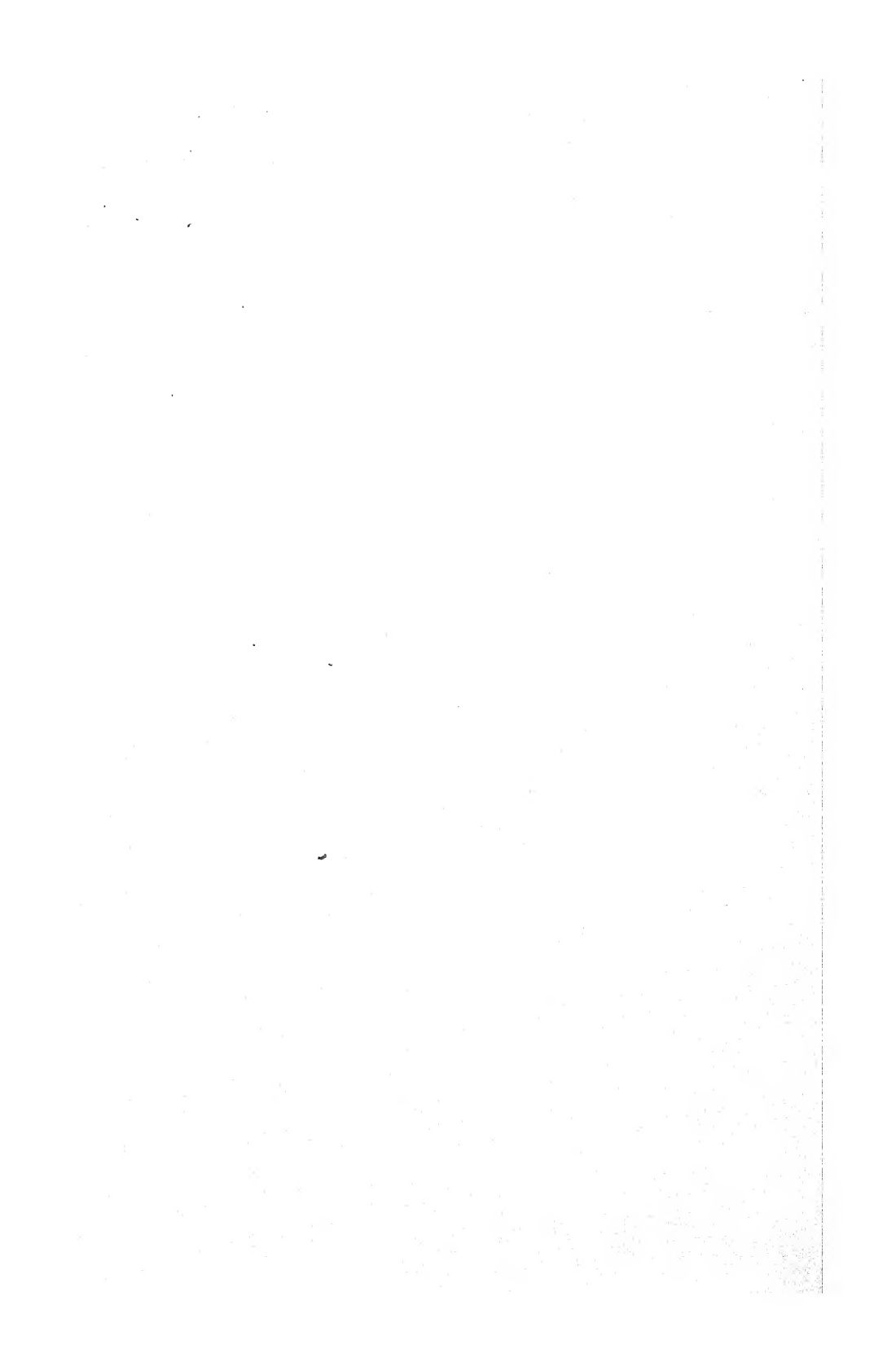
Figs. 8-10. *C. globosum*.

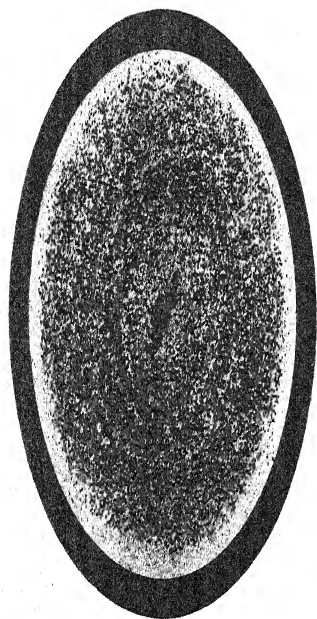
Fig. 8. The parent strain. Perithecia are very freely produced as in the case of *C. cochliodes* (parent strain) the substratum is practically uncoloured.

Fig. 9. Saltant G1. Perithecia are not so plentiful as in the parent and are considerably smaller in size. The substratum is coloured orange chrome.

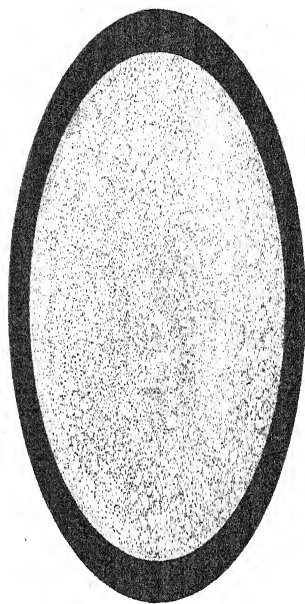
Fig. 10. Saltant G2. This produces a coloration of the substratum very similar to that of G1, but differs from it in exhibiting a strong staling reaction on malt-agar.



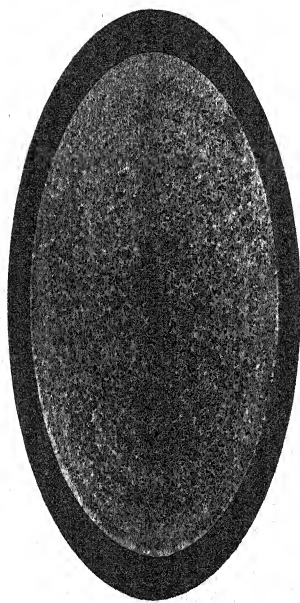




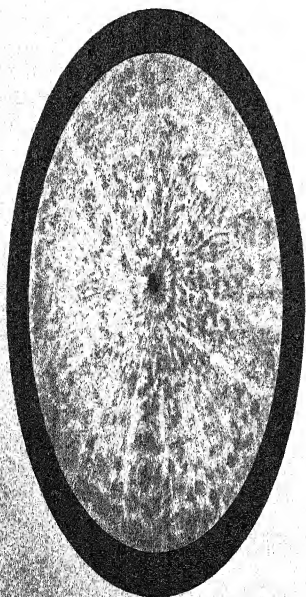
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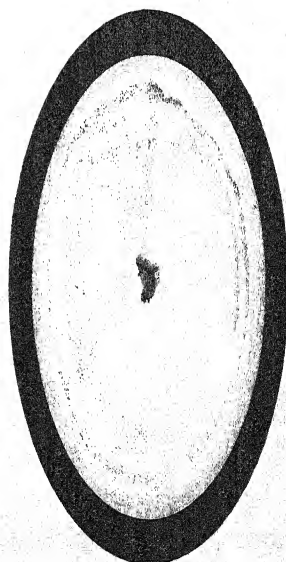
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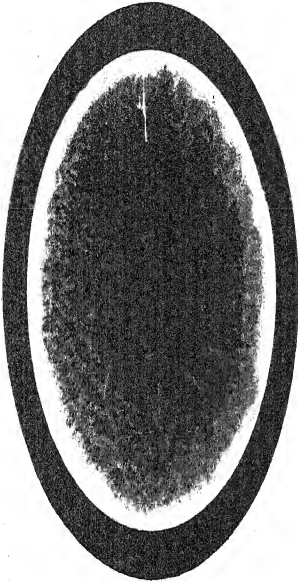
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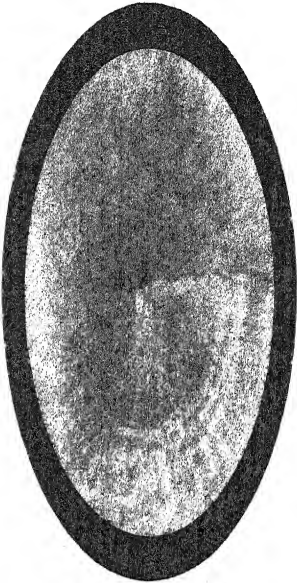
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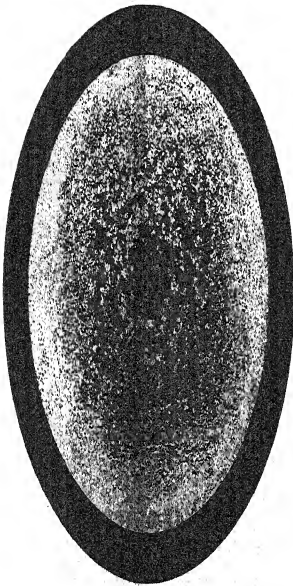
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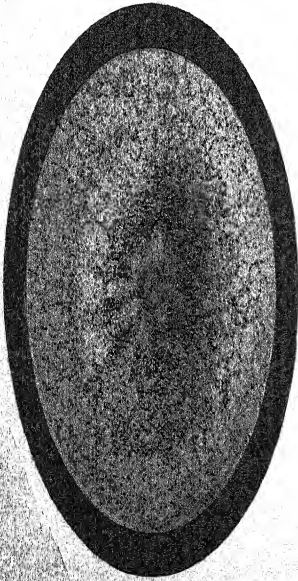
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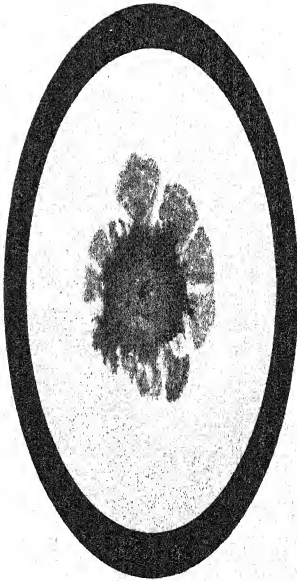
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# The Life-history and Morphology of *Olpidiopsis Ricciae*, nov. sp., Infecting *Riccia* Species in South Africa.<sup>1</sup>

BY

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With twelve Figures in the Text.

## INTRODUCTION.

DURING the winter of 1932, Dr. A. V. Duthie, while engaged in the study of *Riccia* species occurring in South Africa, drew the author's attention to a fungus prevalent in the rhizoids of different annual *Riccia* species (A.V.D. 5006, 5007, and 5118)<sup>2</sup> collected in the Knysna and Stellenbosch districts.

Through the courtesy of Dr. Duthie, sufficient material was placed at the disposal of the author to enable him to study the life-history of the fungus and to establish its identity.

From these studies it would appear that the organism is an undescribed species of *Olpidiopsis*.

## METHODS AND MATERIAL.

Most of the observations recorded were made from sections of living material kept in water on slides, covered by cover-glasses (or in drop culture in van Tieghem cells) for a number of days. The development of the different phases of the fungus were followed by examining the material frequently, with as little disturbance of the sections as possible. Bacterial contamination often became troublesome after a few days, and in many cases obliterated parts of the sections.

*Riccia* plants, infected with this fungus, were therefore washed free from sand, and cultured in fresh tap water. This method had the further

<sup>1</sup> Published by permission of the Principal, Stellenbosch-Elsenburg College of Agriculture of the University of Stellenbosch.

<sup>2</sup> The numbers in brackets refer to the specimens of *Riccia* in the private herbarium of Dr. A. V. Duthie. The species are yet to be identified and described (3).

advantage of maintaining the plants in a living condition for the required period of time on a substrate other than soil. By this means the development of phases of the fungus could be induced, which were otherwise absent.

In order to verify the observations on living and unstained sections, especially as regards the number of cilia on the zoospores, some of the newly cut sections were placed on the slides with a drop of water in which the host had grown. This was spread, dried, and stained. Several stains were tried for colouring the cilia, but it appeared that Muir's Modified Pitfield Method (2) gave the best results when the sections were not fixed in formalin or chromo-acetic acid.

#### THE RELATION OF FUNGUS AND HOST.

The fungus is apparently restricted to the rhizoids of the host (Figs. 1-6). In no instance could it be located in any of the other tissues, though occasionally zoospores and resting zoospores were observed in the epidermal cells. This was, however, probably due to displacement during sectioning, as none of the more advanced stages of the fungus could be found in these cells.

The primary and most common site for the development of the fungus is in the basal cavity of the rhizoids (Figs. 1 and 4), where the developing or mature zoosporangia and oospores are very often crowded upon one another. As this basal cavity of the rhizoid is filled by these structures of the fungus, they are pushed into the rhizoidal tube. The fungus occurs less frequently towards the top of the rhizoid, and mature zoosporangia and oospores are seldom found in this locality.

Both the plain and the peg rhizoids were found to be invaded by the fungus; but, in some cases, it appeared to prefer the plain rhizoids.

Though the rhizoids become infected by this fungus when they are still in the act of protruding, they apparently develop normally and in the same way as the uninfected ones. No distortion, swelling, rupture, or necrotic effects could in any case be detected in any of the infected plants. In some cases, however, the walls of the rhizoids were bulged out on account of the extensive development within them of zoosporangia and oospores.

From these observations, it would therefore appear that the fungus has not any detrimental effect on the normal growth of the *Riccia* species. In this connexion it may be mentioned that several fungi have been recorded as living symbiotically on plants belonging to the family Marchantiaceae (5).

## THE DEVELOPMENT OF THE FUNGUS.

*The zoospores.*

The zoospores of this species of *Olpidiopsis* are usually globular; but may at times be semi-ovoid, owing to a short protrudence at the point of emergence of the cilia (Figs. 10 *a*, *b*). Their contents are finely granular with one large vacuole situated in the centre or towards one side of the spore. The two cilia could at times be seen in unstained living material by careful manipulation and close watching, but their true position could only be established after staining with Muir's Modified Pitfield flagella stain (2). The one cilium is longer than the other. They emerge at the same point and may diverge directly after exit, the one pointing to the front and the other trailing behind (Fig. 10 *b*), as described by Barrett (1) for *O. luxurians*, *O. saprolegniae*, and *O. vexans*. In many cases, however, the cilia run alongside each other for about half the length of the shorter one and then separate. They are approximately  $8.3\ \mu$  and  $17.5\ \mu$  in length.

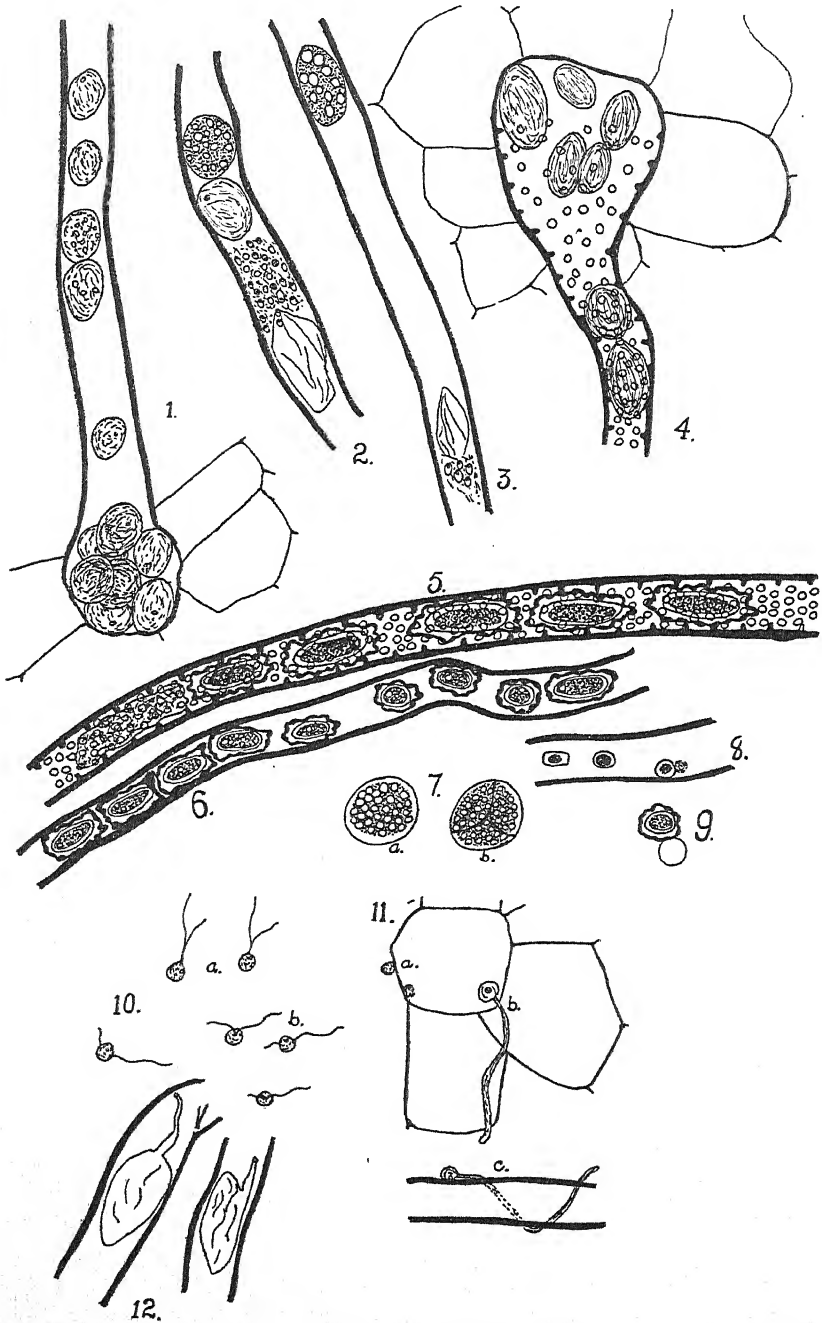
The zoospores possess a marked degree of motility three to four hours before liberation from the zoosporangium, but it could not be ascertained whether they are furnished with cilia while still within the zoosporangium.

The zoosporangium opens at maturity, either by means of an irregular fissure through which the zoospores are ejected within the same rhizoid (Figs. 2 and 3) or by means of a regular beak-like projection (Fig. 12) which opens in the same rhizoid or penetrates its wall. The zoospores are hence liberated in the rhizoid or on its outer surface. In the first-named case, however, they may reach the outside by swimming along in the rhizoid until a rupture, inflicted by mechanical means, is reached through which exit is possible.

When exit from the rhizoid is not possible, the zoospores retain their cilia for only a short period of time, after which the cilia shorten and the spores encyst themselves to develop into zoosporangia or oospores, situated in the same rhizoid as the original zoosporangium.

Where the zoospores are liberated outside the host plant they swim about for a period of two to three hours. The periods of rest and of movement of the zoospores were not closely studied. Periods of rest were frequently observed, but whether this was preceded by a retraction of the cilia and followed by their renewed outgrowth and motion of the spores was not ascertained.

After the above period the zoospores settle down at a suitable point of infection, shorten their cilia, and become surrounded by a wall. Within a period of about two hours the cell-wall of the host is penetrated by short pointed outgrowths of the spores, through which their protoplasmic contents are probably emptied into the cells of the host (Fig. 11 *a*). No actual transgression of these contents could, however, be observed.

FIGS. 1 to 12. *Olpidiopsis Ricciae*, nov. sp.

All figures were drawn with the aid of Abbe's drawing apparatus with an enlargement of 1,000 to 1,200.



Successful penetration apparently takes place only into epidermal cells which are about to grow out into rhizoids. At this stage the outer walls of these cells are probably stretched, and, therefore, easily penetrated by the fungus. Cases have been observed where zoospores have attempted to penetrate the walls of full-grown rhizoids, and other parts of the *Riccia* thallus, but without success.

In drop cultures, of old sections kept under moist conditions for a period of five days, the free swimming zoospores were found to come to rest after a given period of two to three hours, but, in the absence of suitable material to infect, they germinated by means of a germ-tube. This germ-tube is unbranched, slender, 80–100  $\mu$  long and 2.4–3.2  $\mu$  thick (Fig. 11 *b, c*). In the further absence of suitable material to infect, growth of the germ-tube stops, and ultimately the spore and tube dies. Instances have also been observed where this tube curled round older rhizoids, but, however, without any penetration.

### *The zoosporangia.*

The fungus is at times scarcely visible in the rhizoidal cell immediately after infection. It is finely granular until it has become encysted. As it develops into a zoosporangium its contents become denser and the granules enlarge. When the young sporangium reaches the size of about 30–40  $\mu$ , spore differentiation can be detected by the presence of large protoplasmic globules within the sporangial wall (Figs. 1, 2, and 3). In younger sporangia several vacuoles have frequently been observed which ultimately fuse to form a single, large, central vacuole similar to that described by Schwarze (6) for *O. saprolegniae*. The cleavage stages and progressive differentiation of the zoospores was, however, not traced.

Two to three hours before spore liberation the contents of the zoosporangia start a slow revolving movement, which gradually increases in rapidity until the sporangium opens. The zoospores may escape from one mass, but it was observed that the zoospores in some sporangia were grouped into three masses about thirty minutes before spore ejection (Fig. 7 *b*). All three masses were separately revolving and inter-revolving with one another until the opening of the zoosporangium, when each mass again separated into its respective zoospores.

The manner in which the zoosporangia open appears to depend entirely upon the environmental conditions, especially upon the amount of water

FIG. 1. Young zoosporangia in the base and tube of a plain rhizoid. FIGS. 2 and 3. Mature and empty zoosporangia and liberated zoospores in plain rhizoidal tubes. FIG. 4. Young sporangia in a peg rhizoid. FIG. 5. Oospores in a peg rhizoid. FIG. 6. Oospores in a plain rhizoid. FIG. 7 (*a*) Zoosporangium two and a half hours before spore ejection (contents revolving). (*b*) Same zoosporangium fifteen minutes before opening (three sections revolving and inter-revolving). FIG. 8. Various stages in oospore initiation. FIG. 9. Empty antheridial cell attached to a maturing oospore. FIG. 10 (*a*) and (*b*). Biciliate zoospores in stained and unstained sections. FIG. 11. (*a*) Zoospore in the act of penetration. (*b*) and (*c*). Germination of zoospores by germ-tubes. FIG. 12. Beak-like opening of zoosporangia cultured in tap-water.

present. When the infected *Riccia* plants were cultivated in moist soil, the sporangia regularly opened by means of irregular fissures (Figs. 2 and 3). Though a large number of sections were examined of plants grown in wet soil, no evidence could be obtained that the sporangia opened by means of a regular pore, characteristic of the *Olpidiopsis* species. In the species under consideration spores are liberated within the rhizoidal tube, in which they usually again develop into either zoosporangia or oospores, or otherwise they may escape through ruptures, as was frequently evident in sections.

When the infected plants were cultured in clean tap-water for about four days, the mature zoosporangia develop a single beak which may either penetrate the wall or extend for a short distance in the cavity of the rhizoid (Fig. 12). In the first case the zoospores escaped through this opening to be delivered in the water surrounding the rhizoid, whereas in the second case they are again delivered in the cavity of the same rhizoid.

Where the zoospores escape into the same rhizoid, in which the mother zoosporangia are situated, the ultimate result is that the bases and the lower parts of the rhizoids are packed, at times mono- or distichously, with developing and mature zoosporangia and oospores. They are, however, of less frequent occurrence towards the tips of the rhizoids.

By these differences in the manner of spore liberation, the fungus probably ensures its propagation under various conditions. Where water is insufficient for the swarming of zoospores, they are liberated in the rhizoids where their development to zoosporangia or oospores is guaranteed. Where water is, however, present in sufficient abundance, the zoospores will be liberated outside the rhizoid and new infection is very likely to occur.

#### *The oospores.*

They are usually warted, more or less circular to elliptical, but their actual shape depends largely upon the type of rhizoid in which they develop and upon their abundance in these rhizoids. In the peg rhizoids they are usually elongated, and vary considerably in length (Fig. 5). In the plain rhizoids, however, they are more or less circular or otherwise slightly elliptical (Fig. 6), but not elongated to the same extent as in the peg rhizoids. Similar differences in shape were also observed in zoosporangia developing in the two types of rhizoids. When the oospores are closely packed in the host they may be laterally and (or) terminally depressed, being irregularly cubical or cylindrical.

The contents of the oospores consist of two layers of protoplasm, which are clearly visible when stained with Loeffler's flagella stain. The outer layer of protoplasm is finely granular, whereas the inner layer consists of larger granules than the outer.

The oospores of this species of *Olpidiopsis* on *Riccia* species from South Africa develop in apparently the same way as described by Barrett for the various *Olpidiopsis* species he studied ((1) pp. 219-23). The close association of two cells of dissimilar contents, i.e. a larger cell with densely granular contents (oogonium) and attached to it a smaller cell with lighter, finely granular contents (antheridium), has frequently been observed (Fig. 8). No actual transgression of protoplasmic contents from the antheridium to the oogonium could, however, be followed.

The attachment of the empty antheridial cell to the maturing oospore was very seldom found, probably on account of the usual compactness of masses of oospores and zoospores in the rhizoids, in which case the antheridial cells are probably pressed out of sight. The antheridial cells were hence only occasionally seen where oospores were freed from the rhizoids (Fig. 9).

The formation of oospores appears in this case to be in close relation to the resting period of its hosts, all three species of which are annual. These spores are especially abundant towards the end of the winter rains, at which time the host also stops its growth. Their development is in this case more likely to be the result of a change in climatic conditions.

Germination of these sexually formed spores could not be effected, but would probably take place at about the time when the spores of the *Riccia* species germinate at the beginning of the rainy season. The manner of germination is most likely similar to that described by Fischer (4) for *Olpidiopsis* (*Pseudolpidium*).

#### DESCRIPTION OF THE FUNGUS.

##### *Olpidiopsis Ricciae* nov. sp.

Zoosporangia globose or elliptical, smooth, colourless, solitary, or gregarious, formed in bases of the rhizoids or in the rhizoidal tubes, usually  $24.0-40.0 \times 20.0-35.7 \mu$ ; opening by single and irregular fissures or by means of single, unbranched exit tubes. Zoospores globose or slightly ovoid, hyaline  $2.4-4.0 \mu$  in diameter, with two cilia attached to the anterior end. Oospores irregularly globose to elliptical or cylindrical, often laterally and terminally depressed, hyaline, or light brown,  $14.4-48.0 \times 12.8-32.0 \mu$ , surrounded by a thick, warted wall. Antheridia spherical, smooth hyaline. Germination of oospores not observed.

Zoosporangii globosis vel ellipsoideis, levibus, hyalinis, solitariis aut gregariis, in radicibus rhizoidum aut in rhizoidibus tubis formis, plerumque  $24.0-40.0 \times 20.0-35.7 \mu$ , apertis solitariis et inaequalibus fissuris aut solitariis ramis carentibus tubis; zoosporis globosis vel aegre ovatis, hyalinis  $2.4-4.0 \mu$ , in diametribus, duobus ciliis apicibus insertis; oosporis irregulariter globosis ad ellipsoideis vel cylindricis, plerumque lateraliter et terminaliter

depressis, hyalinis aut leviter fuscis  $14.4-48.0 \times 12.8-32.0 \mu$ , cinctis crassis et verrucosis endo- et exosporis, antheridiis sphaericis, levibus, hyalinis.

Hab. in *Riccia* sp. (A.V.D. 5006) (3) Stellenbosch, S.A.

*Riccia* sp. (A.V.D. 5118) Knysna, S.A.

*Riccia* sp. (A.V.D. 5007) Stellenbosch, S.A.

This species of *Olpidiopsis* occurring on *Riccia* species is apparently undescribed and unrecorded, which has led the author to establish it as a new species.

#### SUMMARY.

1. A fungus belonging to the genus *Olpidiopsis* is recorded on three different species of *Riccia* from South Africa.
2. The fungus is restricted entirely to the rhizoids, which are infected when they are still in the initial stage of development.
3. For various reasons the question is raised whether the relation of fungus to host is symbiotic.
4. The zoospores of this fungus are biciliate and are liberated within or on the exterior of the rhizoids, and cause, in the latter case, infection in the same way as reported for other *Olpidiopsis* species. Some cases of zoospore germination by means of a slender germ-tube are also recorded.
5. The zoosporangia mature in the usual way, and, depending largely upon the amount of water present, may open either by an irregular fissure or by a well-developed beak-like opening.
6. Observations indicate the development of oospores to be preceded by the fertilization of the oogonium by an antheridium. Empty antheridial cells attached to the oospores were, however, seldom to be seen.
7. This fungus is named and described as *O. Ricciae* nov. sp.

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# A Disease of Cultivated Mushrooms Caused by *Verticillium Malthousei* sp. nov.

BY

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With Plates XXVI-XXVII and six Figures in the Text.

## CONTENTS.

	PAGE
DESCRIPTION OF THE DISEASE . . . . .	764
1. Association with Insects . . . . .	764
2. Occurrence; External Symptoms . . . . .	764
3. Internal Symptoms. Host and Parasite . . . . .	767
ISOLATION OF THE FUNGUS. . . . .	768
Effect of Heat on Cultures . . . . .	769
INFECTION EXPERIMENTS . . . . .	770
COMPARISON WITH OTHER FUNGI PARASITIC ON MUSHROOMS . . . . .	777
TECHNIQUE OF EXAMINATION . . . . .	782
SUMMARY . . . . .	783

ATTENTION in the past has been paid by mycologists to fungi found growing upon Agarics, but mostly from a systematic point of view. Of those recorded on mushrooms (*Psalliota arvensis* and *P. campestris*), *Mycogone perniciosa*, *Cephalosporium Costantinii*, and *C. lamellaecola* are parasitic.

*M. perniciosa* has been dealt with quite recently by F. E. V. Smith (6) from the historical, cultural, and economic aspects, and by E. B. Lambert (3) in his studies on its temperature-relations. *C. Costantinii* and *C. lamellaecola* are new species established by F. E. V. Smith (6).

The object of the present paper is to present studies on a further disease of cultivated mushrooms caused by *Verticillium* sp., which is here shown to be parasitic, and to compare this fungus with the others already mentioned. The name *Verticillium Malthousei* sp. nov. is proposed.

## DESCRIPTION OF THE DISEASE.

1. *Association with Insects.*

In October, 1929, specimens of diseased mushrooms were received from a grower in Kent; they were small, imperfect in shape, and completely covered with a white, close fungus growth which was not flocculent but gave to the surface a matt appearance. The stipe was swollen and sometimes curved, and the very small unopened pileus so ill developed as to be occasionally hardly distinguishable from the stipe. Both mites (*Acaridae*) and springtails (*Collembola*), which are well-known enemies of mushrooms, were present and the grower was naturally anxious to ascertain whether these or the fungus was the primary cause, since steps would have to be taken to stop the spread of the trouble.

Dead mites and springtails on the surface of the fungus-covered mushrooms (Pl. XXVI, Figs. 1 and 2) were in such numbers as to give rise to the suspicion that the fungus might possibly be a secondary entomogenous species. Microscopical examination showed it to belong to the genus *Verticillium*. Observation of some of the mites and springtails which were still alive, revealed that their movement was being impeded by a large accumulation of mucilage and spores adhering in the form of a globule at the extreme end of each leg. As these animals made their way over the forest of conidiophores on the surface of the mushroom, their load increased until they were brought to a standstill and eventually died. Examination of dead and living specimens confirmed the above observations; in no case were any hyphae seen growing from the animals' bodies, and it was concluded that the association of mites and springtails with the fungus was accidental and due entirely to the holding power of the mucilage which is present in the heads of spores at the tips of the conidiophores.

2. *Occurrence in Commercial Mushroom Houses: External Symptoms.*

The mushroom houses whence the specimens came were visited in the following month. The disease occurred in fifteen houses, each measuring 120 ft. x 20 ft. and each containing five ridge beds.<sup>1</sup> Crops had been grown in these houses since they were built in 1927 and the disease had occurred in 1928 and 1929; it was more prevalent in the summer months, or in winter if the temperature of the houses was raised above 55° F. No connexion could be found between the type of spawn used and the occurrence of the disease, for attacks were equally severe on beds spawned with pure-culture spawn (both white and brown mushrooms) as on those spawned with brick spawn.

<sup>1</sup> Beds made in August cropped from October to February and those made in March, from May to August. No beds were made up in these houses from the beginning of April to the end of July.

Deformed mushrooms were distributed throughout the length of the beds; they were sometimes a shapeless mass or with swollen and curved stalks, sometimes with strips of the tissue peeling and curling downwards, and with small caps, the edges of which were seldom capable of separating from the stipe. They occurred here and there, sometimes singly, but more commonly in clusters and occasionally growing in the same clump or group with normally shaped mushrooms (Pl. XXVI, Fig. 2). They were distinguishable most readily, with only the light of an oil lantern, by their abnormal shape and very white colour which formed sufficient contrast with that of healthy specimens even of the white variety. The beds were coming into full bearing and had been picked over for some weeks. Inspection of mushroom clumps showed that the disease was commonly present at the base where several buttons were attacked and were covered with the white fungus. From any diseased button in contact, the fungus appeared capable of attacking and spreading to the stipe or pileus of larger and otherwise healthy mushrooms in the group. The upward or downward spread on the stipe of normally shaped and more mature mushrooms, of which the gills were wholly or partly exposed, was more rapid than the lateral spread; consequently longitudinal sunken streaks covered by the parasitic fungus occurred on the stem. Specimens were seen in which the spread had been sufficiently rapid to reach the edge of the pileus before it separated from the stipe, the *Verticillium* growing on the upper surface of the pileus as well as on the stipe. It was evident that when the disease spread up one side of the stipe and passed over the edge of the pileus at a stage before the veil was distended, the expansion of the pileus and the stretching of the veil were prevented at that place on the circumference. The pileus therefore became asymmetrical and was sometimes tilted. In these cases of infection of mushrooms, presumably after they were more or less mature, the parasitic fungus was greyish white in colour and the parts attacked were slightly sunken. Separate areas or spots of infection, greyish white in colour, occurred on the upper surface of the pileus of some nearly mature mushrooms without there being any visible infection of the stipe. In commercial practice, picking is carried out at frequent intervals and no mature mushrooms are left on the beds; consequently in the present instance it was not possible to see the later effects of the disease. Moreover, for some time previously, diseased specimens had been removed as fast as they appeared. The most deformed and fungus-covered mushrooms were firm and somewhat leathery and were not rotting. In the light of day it was seen that the white fungus covering was, in parts, often tinged with a faint grey-brown or grey-lilac colour, though this might be confined perhaps to one side only of the swollen stipe or of the shapeless mass representing the mushroom.

Examination in the laboratory of diseased but well-formed mushrooms

of which the veils were broken, showed that the *Verticillium* was producing conidiophores also on the sides and edges of the gills. This infestation was far commoner in proximity to the part of the stipe or pileus-edge attacked and was attributed to mycelial spread; it was also to be found in isolated patches on any part of the gill area, and in such cases it originated probably from insect-borne spores. The amount of the fungus on the gills was not always sufficient to be seen without the use of a microscope, but occasionally the gills were completely covered with a white growth or with separate greyish white colonies which were easily visible. Dense masses of long verticillate conidiophores projected from the gills, and hyphae passed across from one gill to the next. Apart from the hindrance to, or suppression of development of gills, no damage such as wet rot or distortion resulted, nor was there commonly any fasciation such as that figured by Smith ((6) (Pl. V, Fig. 9) in the case of *C. lamellaecola*. At a later period it was found that fully expanded mushrooms, otherwise completely healthy, might become infected on the gills; indeed, the gills proved extremely susceptible to attack, and colonies were seen growing in wavy white lines traversing the gill-edges, and presumably in the path taken by *Sciara* flies<sup>1</sup> which had walked on the under side of the pileus. In a very few cases, in the inoculation experiments described below, where mushrooms had been left to mature on the beds after having become infected, the *Verticillium* radiated from a streak on the stipe and occupied a complete sector of the gill area, making the gills in that sector wholly grey-white in colour, puckering them and joining them together. The gills were then ill developed towards the periphery and the pileus on that sector was turned down at its edge, appearing as though restrained from expansion. The diameter of the pileus in these specimens was as much as 9.8 cm.; the roughly triangular infected sector of gills was 2.5 cm. wide and 2.5 cm. long. Inoculation experiments with cut mushrooms easily reproduced the sectoring effect (Pl. XXVII, Fig. 5) in addition to the longitudinal sunken streaking of the stipe. It was found that the fungus was able to spread from the gills and cause a sunken streak on the stipe; vice versa, it could spread from the stipe and cause a white sector on the gills.

The *Verticillium* was not found occurring naturally on the gills of young mushrooms, having the veil unbroken, but already so well formed that the pileus-edge was nearly separating from the stipe; it was, however, present on the immature and unexposed gills of the very deformed specimens which obviously had been infected from the youngest stage of growth and in which the pileus would never develop so far as to break away from the stipe.

<sup>1</sup> *Verticillium* spores were found adhering to the legs of flies which were removed from diseased mushrooms.



3. *Internal Symptoms. Host and Parasite.*

Deformed mushrooms with swollen or curved stipe and much reduced pileus and with a grey-white covering of *Verticillium* were examined internally. When cut longitudinally through the medulla, they commonly showed a narrow grey-brown zone to a depth of 1 to 2 mm. below the conidiophore-infested surface, but the rest of the stipe or pileus internally was white. In mushrooms, which evidently had been long infected, the brown coloration extended deeper and, in some, the whole internal tissue was greyish brown. The discoloured parts were comparatively dry and of more felt-like consistency than normal healthy tissue; even in mushrooms with a white external covering of the parasite and completely discoloured internally, there was no wet rot. This fact, in contrast to the rapid decomposition brought about by *M. perniciosa*, was clearly demonstrated when diseased mushrooms were kept in closed glass dishes in a cool room for a month or two at a temperature of 50° to 55° F. and remained in good condition.<sup>1</sup> Even in warm weather, with a room temperature of 65° F., diseased specimens were kept without becoming decomposed, e.g. from July 22 to August 4.

An interesting feature of the deformed mushrooms was that they were rarely solid. A small or large cavity<sup>2</sup> (up to  $\frac{1}{4}$  in. diameter) was usually to be found near the junction of pileus and stipe, and occasionally other cavities occurred where the brown infected tissue had become torn internally by growth changes. These cavities proved of considerable value in facilitating the subsequent work of isolating the fungus, for though they had no external opening they were lined with conidiophores and hyphae passed across from wall to wall.

Sections of diseased mushrooms were stained with aqueous Bismarck Brown. The mushroom hyphae were large<sup>3</sup> and agreed with the measurements made by Smith (6, p. 86), whereas the *Verticillium* hyphae were considerably narrower, in this respect resembling the hyphae of *Mycogone*; they measured 1-3  $\mu$ , or 4-5  $\mu$  at a place of branching and were not stained

<sup>1</sup> Occasional specimens became rotten with bacterial putrefaction after being kept for fourteen days; they smelled strongly of ammonia.

<sup>2</sup> In normal mushrooms, after a certain elongation of the stipe, a cavity is often present where the centre or upper or lower end of the medulla has parted. This cavity may be as much as 2.0 cm. long and 0.4 cm. wide.

<sup>3</sup> Measurements of mushroom hyphae were made; they were found to vary within the following limits:

Stipe	{ inner cells (medulla)	4 $\mu$ to 28 $\mu$ loosely woven.
	{ middle cells (cortex)	4 $\mu$ to 26 $\mu$ closely woven.
	{ outer cells (exterior)	4 $\mu$ to 14 $\mu$ very closely woven.
Gills	{ inner cells	4 $\mu$ to 24 $\mu$
	{ outer cells	2 $\mu$ to 12 $\mu$
Pileus	{ inner cells	6 $\mu$ to 30 $\mu$ loosely woven.
	{ outer cells	5 $\mu$ to 10 $\mu$ closely woven.

by Bismarck Brown, thus being easily differentiated from the mushroom hyphae which (in the pileus region) measured commonly  $16\mu$  in diameter, and were stained brown. In deformed specimens the parasitic hyphae were present not only in the discoloured tissue, but also in that which remained white; in well-formed mushrooms which showed localized areas of attack, the hyphae penetrated only a short way beyond the limits of the internal discoloration. When malformed diseased mushrooms were cut across and kept in a closed dish for about 24 hours, a growth of *Verticillium* conidiophores appeared on the cut surfaces. In connexion with the proximity of diseased and healthy mushrooms, it is of interest to record that sections cut through the place of union of deformed infected mushrooms and healthy ones, where the stalks were actually united at the base (as in Pl. XXVI, Fig. 2), showed no internal invasion of hyphae from the infected stipe into the healthy one.

Hand sections of infected gills showed hyphae present in the trama, between the basidia, and also externally. In gills with a considerable amount of the fungus visible externally, complete spread through the gill had not always taken place, the parasitic hyphae being somewhat localized. In the most advanced cases of gill-infection, however, a complete permeation by the parasitic hyphae occurred.

#### ISOLATION OF THE FUNGUS.

Isolations were made without difficulty (*a*) by plating out pieces of diseased mushroom cut out after surface-sterilization with a hot scalpel, and (*b*) by plating out dilutions of spores in sterilized water. The collection of spores, likely to give pure cultures, from the surface of a mushroom necessitated finding a crevice or protected place in which there would be some chance of freedom from contamination by moulds or bacteria. When it was found (see above, p. 767) that infected deformed mushrooms commonly contained a cavity inside the stipe in which the fungus produced conidiophores, isolation was greatly facilitated. Examination of mites on naturally infected mushrooms had shown that masses of spores and mucilage adhered to their feet.<sup>1</sup> Cultures of the fungus, free from all contamination, were obtained by placing mites taken from the gills of infected mushrooms, in the centre of agar plates and allowing them to crawl on the surface of the medium. The original isolations were made on prune agar and subcultures on coconut agar.

<sup>1</sup> A white globule of spores and mucilage, three or four times the thickness of the animal's leg, was attached to each foot. The viscous nature of the globules was observable with the microscope when a mite was left inverted on a slide. In its attempts to turn over, and with its legs waving in the air, the mite soon became immobile when the globules made contact and fused into one mass. Only after a struggle was the animal able to separate its feet.

By placing a spore-loaded mite in a drop of water, thousands of spores were set free but a gummy mass remained on each foot for some time, although in contact with water.

Single-spore cultures were made with spore-suspensions in sterile water and with subsequent dilutions in potato-gelatine, by the poured plate method. The spores in the most diluted plate were allowed to germinate for 36 hours at 17°–19° C. and after the plate had been examined at all depths, certain single germinating spores were cut out and removed to fresh plates of potato-gelatine. Other single spores, previously marked, were cut out after germination had proceeded for 60 hours. In all, eight single-spore cultures were made and from these were derived all the cultures used in the subsequent inoculation experiments and for measurements and description of the fungus.

Growth was good and fairly rapid on prune-, coconut-, potato mush- and potato extract-agar and also on sterilized manure compost such as is used for mushroom beds. On the surface of the agar, at 20° C., a close white mycelium was formed; no change of colour occurred except to a greyish tint when spore-production was at its maximum on potato-agar and, excepting torulose hyphae, no sclerotia, chlamydospores, or other resting or reproductive bodies, in addition to the usual conidial masses were observed. When mixed cultures<sup>1</sup> were made with *Mycogone perniciosa*, there was no repulsion (see Plate XXVII, Fig. 6).

#### *Effect of Heat on Cultures.*

Investigation of the thermal death point was carried out by the method used by Lambert (3, p. 77) for *Mycogone perniciosa*. As a preliminary test, the fungus was exposed for varying periods to only one temperature, i.e. 40° C. (= 104° F.), this being chosen as representing one of the lowest temperatures likely to be encountered by the fungus on the supposition that it may reach the mushroom beds by way of the manure.<sup>2</sup> Freshly made coconut agar slopes were placed in an incubator with an internal temperature of 40° C. By means of thermometers placed in some of these tubes it was found to require about 2 hours to warm the agar from 20° C. to 40° C. When this temperature had been reached, the tubes were placed in a water-bath at 40° C. while awaiting inoculation. Transfers of large blocks of agar (about 1 cm. ×  $\frac{3}{4}$  cm. ×  $\frac{1}{2}$  cm.) from cultures of the fungus were then made, and the fresh agar slopes promptly placed in the incubator at 40° C. For any one period of exposure four slopes were included in the test. As controls, four transfers were made to slopes at 40° C. which were then allowed to cool, and four transfers to slopes which were already cool. Beakers of water were placed in the incubator and all tubes were

<sup>1</sup> The behaviour of the fungus in mixed culture with *Mycogone perniciosa* was used by Smith (6, p. 93) as a means of distinguishing *Cephalosporium Costantinii* from *C. lamellaecola*. The former showed no repulsion.

<sup>2</sup> In the process (lasting from 1 to 3 or 4 weeks) of making the compost for the beds, the manure is turned at least three times and reaches a temperature between 140° and 160° F. for days at a time.

sealed with rubber caps. Each group of four tubes was withdrawn from the incubator after exposure to a temperature of 40° C. for 1, 2, 3, 4, 5, 6, 9, 12, and 24 hours, and the tubes were then kept at 20° C. This experiment was repeated four times and each series of 44 tubes (including eight controls) was kept for one month. In no case was there growth in any of the tubes exposed for 6 hours or longer, whereas in the control tubes and in those exposed for shorter periods, growth of the fungus took place. After 1 to 3 hours exposure the fungus was capable of growth in all four tubes; after 4 hours and 5 hours exposure, it was capable of growth in only some of the tubes and a loss of vigour was evident.

The possibility remained that some alteration of the medium might have occurred in those tubes which had been kept for the longer periods at 40° C. This, if it did occur, might perhaps account for the absence of growth. To investigate the matter, cool agar slopes were inoculated after they had been exposed for the longer periods at 40° C., and in all cases a normal vigorous growth resulted, showing that the medium had not been affected by the prolonged heating.

The result, like that obtained by Lambert, is of practical importance in indicating that, apart from insects, the casing soil rather than the manure of the beds is to be suspected as a means of introduction when the disease appears in mushroom-growing houses.

#### INFECTION EXPERIMENTS.

##### 1. *With mushrooms on ridge-beds.*

Owing to the impossibility of securing at short notice a bed of mushrooms in full cropping, available for inoculation experiments, and situated away from the scene of the original occurrence of the disease, preliminary work was begun in January, 1930, in one of the mushroom houses already infected. Mushrooms of the white variety in various stages of development<sup>1</sup> were selected, and different methods of inoculation were tested. All individuals or clumps of mushrooms inoculated were covered with inverted flower pots to keep the surrounding atmosphere more humid.

On January 27, fragments of agar culture, five weeks old, were inserted in shallow wounds made in five separate mushrooms which were in different stages of development; two in the button stage were chosen for inoculating the young pileus and three, further developed, for inoculating the stipe, the veil, and the more expanded pileus. Examination one week later showed a sunken area (6 mm. to 12 mm. diam.) on the pileus of the first

<sup>1</sup> The smallest buttons usually break through the casing soil in groups and in close contact and thus make difficult the marking of very young individuals. For this reason, and because it was found impossible to prevent drops of water carrying the inoculum from being absorbed by the casing soil in contact, the smallest visible mushrooms were inoculated in all later experiments by applying the liquid also to the soil surrounding them.

two, with the fungus spreading from the edges of the wound. At this date (February 3) both the pilei measured 4 cm. in diameter. On the third mushroom, the fungus was spreading from a wound at the base of the stipe and was covering an area of 6 mm. diameter; on the fourth, inoculated in the centre of the upper surface of the pileus, no spread from the place of inoculation had taken place; on the fifth, inoculated by inserting the agar within the veil, no infection was visible. The third and fifth, being fully grown on February 3, were removed and kept for four days at room temperature (60° F.) by which time the third showed an infected area 1.5 cm. diam. around the scratch and the fifth an area 5.0 cm. wide extending on to the upper surface, of the pileus from the remains of the veil at the place of inoculation and considerable infection of the gills. The remaining mushrooms (nos. 1, 2, 4) were left to grow until February 10 and were then removed because mature. No further spread of the fungus had occurred on nos. 1 and 2 and in no. 4 there was growth only in and at the edges of the wound, affecting an area of 0.75 cm. in the centre of a pileus of 7.0 cm. diameter.

In the above experiment, no control mushrooms on the beds were wounded with a sterile instrument; the intention had been in the first place to reproduce the deformity which was so characteristic of the disease and in this the wound inoculations all failed. At the temperature of the mushroom house (about 55° F.) the fungus was able, in all five cases, to establish itself on the growing mushroom tissue, but it was evident that the rate of growth of the parasite was not sufficiently fast to cause deformity of mushrooms which were already at or past the button stage. Subsequent experiments in which mushrooms, as small buttons, were inoculated by wounding and by atomizing with spore-suspensions, confirmed this conclusion.

A second method of inoculation was tried on January 27 in the same mushroom house. A single drop of a spore-suspension in sterilized water was placed on each of eleven isolated small buttons of 12 mm. diameter which had only just broken through the casing soil. On each of seven similar mushrooms, as controls, a drop of sterile water was placed. One week later, on February 3, four of the inoculated mushrooms which showed a brown area (1.5 — 2.0 cm. diam.) on the pileus, on which the fungus was fruiting, were removed and kept in the laboratory at 59° F. for four days. At the end of this period, the infected areas had increased and the patches of *Verticillium* measured from 3.5 cm. to 6.0 cm., completely covering the upper surface of the pilei. Two mushrooms showing infected areas on the pilei on February 3 were left unpicked, together with five which showed brown areas on which the *Verticillium* was not distinguishable. These seven were gathered and examined on February 10 when all showed the presence of the fungus on areas of 1.25 cm. to 3.0 cm. diameter on pilei measuring 4.25 cm. to 8.3 cm. diameter. Six of the control mushrooms

remained healthy until February 10, the pilei measuring from 4.0 cm. to 6.5 cm. diameter, but the seventh became infected.

A third method of inoculation, without controls, was tried on January 27. Sterilized water was added to two tube cultures, and after being shaken to make a spore-suspension, it was poured on two groups of very small buttons which were just forcing their way through the casing soil. When examined on February 3, one group consisted of 22 mushrooms, and the other of 9, and both appeared healthy. On February 10, however, both groups contained diseased mushrooms. In the first, with 10 healthy, 8 were infected either on the lower part of the stipe or on the upper surface of the pileus or on both, 2 were still more infected, the *Verticillium* fruiting on the upper surface of the cap and running in a straight band on one side of the stipe, and on to the gills along which the fungus extended in a sector to the extreme edge of the pileus. The remaining two were sclerodermoid, white, and completely covered with *Verticillium*. In the second group, 4 mushrooms were infected on the pileus and at the base of the stipe, and the remaining 5 were sclerodermoid, with the fungus mostly on the swollen stipe. After being kept at room temperature for two days, all 9 mushrooms became completely covered with the fungus.

A further series of inoculations were made on February 3. Six young mushrooms were wetted on the pileus with spore-suspensions. After 14 days they were examined, and *Verticillium* was found growing on five of the inoculated pilei; the sixth was healthy. Ten similar mushrooms, moistened with sterilized water as controls, showed eight healthy, but two had become infected. On February 3, three very small and isolated buttons as well as the surrounding casing soil, were well watered with spore-suspensions. One remained healthy, the second was attacked by *Verticillium* on the white rhizomorphs at the base of the mushroom, and the third became attacked on the pileus.

## 2. *With cut mushrooms.*

In the laboratory, experiments were carried out with large numbers of freshly gathered mushrooms which were kept in glass dishes at 15° C. (= 59° F.). The methods of inoculation were varied. Pieces of agar from cultures of the fungus were placed in shallow wounds cut in pileus and stipe, or were dropped on to the gills through a hole cut in the veil. Water suspensions of spores from cultures were also placed on the unwounded surfaces of pileus, stipe and gills. In all cases the inoculations were successful; the *Verticillium* grew in and from the wounds, and on the inoculated unwounded surfaces, in such profusion as to be visible to the naked eye after 3 or 4 days (see Pl. XXVII, Fig. 5). All controls with sterilized water or sterilized agar in place of the inoculum remained healthy. The fungus was re-isolated without difficulty.

The above-described experiments carried out in a mushroom house which already contained the disease and those with gathered mushrooms in the laboratory, owing to the environment, were not entirely satisfactory. They had shown that inoculation of mushrooms, although quite small, resulted only in infections which caused no restriction or deformity of growth and that the deformed mushrooms, so typical of the disease, were only produced by infection at a very early stage of growth, viz. by watering the soil with a spore-suspension before the smallest mushrooms had forced their way through the casing.

### 3. *With mushrooms on a flat bed.*

A mushroom bed was laid down in a shed at Wye, away from all possibility of contamination by the fungus. It was spawned with pure-culture spawn and with brick spawn on January 18, and was producing mushrooms on March 24. Spore-suspensions from cultures of the *Verticillium* were prepared in sterile distilled water on April 8 and, by means of an atomizer, 15 cc. were sprayed over the surface of each of five groups of minute mushrooms which were just appearing over the places where the spawn had been planted. Cardboard shields were used to prevent any of the spray being carried beyond the area it was intended to moisten. Five similar groups of mushrooms, appearing close to those inoculated, had previously been atomized with sterilized distilled water to serve as controls and a further 8 groups, not atomized with water, provided additional controls. The inoculated and control areas were covered with large inverted flower pots to reduce any risk of aerial spread or insect carriage of the disease and to maintain a moist atmosphere. Temperatures<sup>1</sup> were observed by means of thermometers in the shed and at a depth of 2½ in. in the mushroom bed.

The first effect of the inoculation became visible 17 days later, on April 25, when a mature mushroom was found infected on the upper surface of the pileus, with the *Verticillium* fruiting freely. Thirty-one days after inoculation it became evident that the small and very white mushrooms present on all the inoculated areas were of distorted growth. The bed was kept for a period of 2 months and was destroyed on June 13. From the 5 inoculated areas 28 diseased mushrooms and 7 healthy ones were gathered; from the 5 control areas, 71 healthy and 2 infected mushrooms were obtained, and from the 8 additional control areas which had not been atomized, 67 healthy. The two infected mushrooms found in

<sup>1</sup> The temperatures recorded were as follows:

1930	Air temp. (° F.)			Bed temp. (° F.)		
	Max.	Min.	Mean of daily readings.	Max.	Min.	Mean of daily readings.
April	67	39	54	57	48	52
May	70	44	58	64	53	58
June	72	54	66	65	60	64

one of the control areas closely adjoining the fifth area inoculated, were only slightly attacked; on one an area (8 mm. diam.) in the centre of the upper surface of the pileus, and on the other several small areas (5 mm. diam.) on the upper surface of the pileus were the only places of infection. Although every effort was made to suppress insect life in the shed, this slight infection of one control area may have been brought about through the agency of flies or by the process of sprinkling the beds. Twenty of the 28 diseased mushrooms on the inoculated areas were deformed or shapeless, and three types are shown in Plate XXVI, Fig. 3. The pileus was scarcely differentiated, the stipe was either swollen (Plate XXVI, Fig. 3, right) or much elongated (Pl. XXVI, Fig. 3, centre), and peeling back of parts of the stipe was a common symptom. In this phenomenon the free end of the strip of stipe was always uppermost, and the peeling strip not so thick or so broad here as lower down at the end connected to the stipe. All the deformed mushrooms were of a greyish white colour and were covered completely with *Verticillium* conidiophores; in shape, many of them were similar to the sclerodermoid deformities caused by *Mycogone perniciosa*.

#### 4. *With mushrooms in boxes.*

To provide further material for inoculation experiments, 15 wooden boxes measuring each about 20 × 14 in. and 12 in. deep, were spawned with pure cultures of the white variety of mushroom on February 19, 1930. These were kept under dry conditions for a time, and were watered at the end of May when the mycelium had filled the boxes. They were kept on shelves in a greenhouse stokehole which had been out of use for at least 15 years, and which was situated below the ground. The temperature, though fairly constant, was above the optimum for mushroom growing. The extremes of the air temperature minima were 56° F. and 66° F. and the mean of the minima 60° F. The extremes of the maxima were 60° F. and 70° F. and the mean of the maxima 65° F. Readings were taken every 24 hours. Cropping began on June 5 and was allowed to proceed until July 3 to be certain that no disease was present; insects were dealt with by vaporizations of nicotine.

On July 3 and July 5 inoculation of certain boxes was carried out by means of an atomizer, using spore-suspensions from subcultures of the original single spore isolations in sterile distilled water. In each of the boxes numbered 1, 9, 12, 14, only a single group of minute mushrooms was atomized. The group was just visible through cracks in the casing soil, and the largest mushrooms were no more than 1.0 cm. in diameter. This localized inoculation of young mushrooms was carried out in the first place to determine whether any rapid spread of the disease throughout the box would follow, and in the second place to attempt to secure further specimens of distorted mushrooms such as had already been produced in



the last experiment, In boxes 3 and 10, half the area of the casing soil was atomized; the groups of mushrooms breaking through were in the youngest button stage, and the pilei were not more than 1.0 cm. in diameter. In box 2 one third of the area was atomized, and in box 8 the whole area. As controls, boxes 4, 5, 6, 7, 11, 13, 15, were not inoculated, but were placed so that they alternated with the boxes atomized. Before the inoculations were performed, all areas on all the boxes which were not about to be sprayed with spore-suspensions, including the controls, were atomized with sterilized distilled water.

The first effects of inoculation were visible in 3 days and consisted of a light, almost golden-brown, spotting of the white pileus. On the 5th day, when under conditions of high temperature the mushrooms were already nearly fully grown,<sup>1</sup> the infected areas on the pileus were still of light brown colour, but were more distinct (Pl. XXVI, Fig. 4). On these brown areas a thin greyish white covering of *Verticillium* was present, which increased considerably in density when the mushrooms were gathered, and were kept in a moist atmosphere in a closed glass dish over night. The diameter of the greyish infected areas varied from 0.2 cm. to 3.5 cm.; they were slightly sunken but comparatively shallow; no internal discoloration of the mushroom tissue was found at a depth greater than 3.0 mm., and the hyphae penetrated no deeper. From 6 of the spotted mushrooms in box 3, which are shown in Pl. XXVI, Fig. 4, the fungus was re-isolated. From the four groups, all of which had been inoculated on July 3, and from the four larger areas, all of which were inoculated on July 5, 35 infected mushrooms were gathered on July 10. No healthy ones were present. On the same day from the control areas, including the totally uninoculated boxes, 46 healthy mushrooms were gathered and no diseased ones were present.

A few days later, among the further crop of mushrooms reaching maturity and showing the usual symptom of spotting on the pileus, there appeared several others evidently deformed or distorted and covered with a white downy growth of *Verticillium*. On July 22 all mature mushrooms were gathered, viz.—from the inoculated groups and areas, 42 infected and 13 healthy, from all control areas and boxes, 117 healthy and none diseased. Of the 42 infected with *Verticillium*, 20 were deformed or distorted and 22 of normal shape but with spotting on the pileus. During the period July 3 to July 22, the temperature minima had been between 56° F. and 66° F. The deformed mushrooms varied greatly in appearance; some were no more than globular white masses with no differentiation of pileus from stipe but, in the majority, the form of the pileus was clearly distinguishable though much reduced. The swollen and often bent stipe

<sup>1</sup> As has been pointed out by Lambert (3), the period required by mushrooms to reach maturity from the smallest size, varies with the temperature. With the 'Snow White' variety at 21° C. (70° F.), 6 days are required; at 15° C. (60° F.) 10 days; at 10° C. (50° F.) 22 days.

showed the usual symptom of peeling back, or even splitting. In several specimens, where the pileus was severely attacked, the surface had become irregular or lumpy owing to the formation of rounded warty intumescences and depressions. In mushrooms which had developed sufficiently for the gills to be exposed, a greyish white covering of *Verticillium* was present on these also.

On August 2, 24 diseased specimens with discoloured areas on the pileus, 14 deformed and 10 healthy, were gathered from the inoculated groups and areas. From the control areas and boxes 148 healthy mushrooms were gathered and 4 infected. This occasion, one month after the date of inoculation, was the first on which any spread of the disease had taken place. Three of the four infected mushrooms occurred in box 12, in which a single group had been inoculated, and the remaining one in box 2, of which one-third of the area of casing soil had been inoculated. During the growth of mushrooms it is the practice to sprinkle the casing soil with water from the rose of a watering-can, and this having been done in the present experiment, though never until after diseased mushrooms had been picked, the spread may well be accounted for. It was observed that the *Verticillium* spores were readily carried by the legs of flies and, though flies were continually suppressed by the use of nicotine vapour, they must be regarded as possible vectors.

Only one further case of infection by the fungus was found on any control area up to August 29. This occurred in box 10, half the area of which had been inoculated, on August 15. On and after August 29, a few more diseased specimens were found on uninoculated areas, and even the boxes 4, 5, 6, 11 did not escape infection, although no part of them had been inoculated; the following are the numbers of diseased mushrooms found on the uninoculated areas: box 4, 1; box 5, 6; box 6, 3; box 11, 2. The remaining uninoculated boxes, 7, 13, 15, which produced respectively 20, 56, and 40 mushrooms, showed no trace of infection throughout the experiment which was brought to an end on September 13.

From the date of inoculation, until September 13, 455 healthy mushrooms and 28 infected were gathered from the uninoculated areas and boxes. In the same period, 143 diseased and 26 healthy were obtained from the groups or areas and from the one complete box inoculated. With this result, showing 94.2 per cent. of the mushrooms healthy on the control areas and 84.6 per cent. diseased on the inoculated areas, evidence is provided that the fungus is parasitic and apparently very active at the higher temperatures of mushroom-growing.

#### 5. *Control measures.*

The disease is apparently not common, for in the great mushroom-growing districts of Sussex and parts of Kent, it has only once been met

with. There is, however, a possibility that growers may have confused it with the widespread disease caused by *M. pernicioso*.

From the observations already made, control measures should include the suppression of insect life in mushroom houses, the reduction of the air temperature to 50°–55° F., hard picking and removal of all diseased specimens in separate operations and, lastly, the sprinkling of beds only after the diseased mushrooms have been removed. A preventive measure, it is suggested, is to dig the casing soil from below the surface or to steam-sterilize it.

#### COMPARISON WITH OTHER FUNGI PARASITIC ON MUSHROOMS.

As has been described above, the *Verticillium* hyphae are found penetrating between those of the mushroom. Sections cut through the most diseased parts showed that some decomposition of the mushroom hyphae occurs when they are in close contact with the parasite; the walls are less distinct and some hyphae are collapsed. Two distinct kinds of symptom occur: (1) localized spotting of the pileus, gills, or stipe of already well-formed mushrooms. The spotting may increase to form larger areas or, on the stipe, to form longitudinal depressed streaks. (2) Deformity of mushrooms resulting from very early infection. On the surface of those mushrooms which are deformed, as well as in any internal cavities that exist, the *Verticillium* forms conidiophores consisting of long aerial hyphal branches bearing secondary branches in whorls (Text-fig. 3). The mycelial covering of the mushroom is white before spore-production becomes general, but it later becomes greyish.

Diseased specimens are to be distinguished from those attacked by *M. pernicioso* by several diagnostic characters. Both may be deformed and covered with a white mycelium, but that of *Mycogone* is more vigorous and flocculent, and does not become greyish-white even when chlamydospores are being formed.<sup>1</sup> The *Verticillium* mycelial covering is close and almost downy. Peeling of strips of the stipe in a downward direction is a common feature in advanced infection by *Verticillium*, which is not met with in mushrooms attacked by *Mycogone*. Decomposition of mushrooms infected with this *Verticillium* is not rapid, and is not preceded by the exudation of drops of brown liquid which have given to the *Mycogone* disease the name of 'Bubbles' or 'Weeping Disease'.

Microscopically, the two diseases are also readily distinguished; the presence of chlamydospores on the surface and embedded in the tissue of mushrooms is diagnostic of the *Mycogone* disease. The so-called *Verticillium* stage of *Mycogone*, which precedes the general formation of

<sup>1</sup> In culture, the mycelium of *M. pernicioso*, white at first, is light brown in colour when general chlamydospore-production has started. The growing colony is surrounded with a white fringe of hyphae (Pl. XXVII, Fig. 6) on which the *Diplodadium* conidiophores and conidia are borne.

chlamydospores, is the only one which is liable to be confused with the present *Verticillium* owing to the arrangement of the branches<sup>1</sup> in whorls. This conidial stage of *Mycogone* is, however, to be regarded as a *Diplocladium*, for the spores are one-septate<sup>2</sup> at maturity, and are borne singly at the tips of the branches of the verticillate conidiophores without becoming aggregated into masses or conglomerates by the help of mucilage;<sup>3</sup> the single *Diplocladium* spores, again, are much larger<sup>4</sup> than the spores of the present *Verticillium* (Text-fig. 1). The very conspicuous chlamydospores of *Mycogone* constitute the best distinguishing character; their organic connexion with the *Diplocladium* stage is easily observed; they are sometimes borne on one branch of a whorl in which the other branches bear *Diplocladium* spores, but they are more commonly borne on branches which are not part of a whorl.

The present *Verticillium* is also to be distinguished from *Cephalosporium Constantinii* F. E. V. Smith, which has smaller spores ( $3\mu - 7\mu \times 1\mu - 1.5\mu$ ). The conidiophores of *C. Constantinii* arise singly at irregular intervals on the hyphae, and are continuous or occasionally cut off at the base by a septum. In *Cephalosporium* they are unbranched, and it should be noted that the following words from Smith's description of his fungus refer to the hyphae as distinct from the simple conidiophores: 'The branching is frequently subverticillate in the upright portions'. In

<sup>1</sup> The branches composing the whorl number 2-9, most commonly 5.

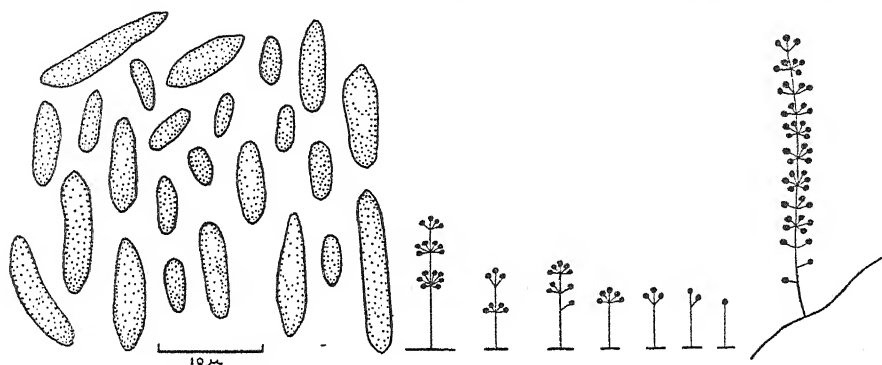
<sup>2</sup> Among several thousands examined, only two spores were seen which had more than one septum, viz. two and three respectively.

<sup>3</sup> Examination by the present writer of large numbers of *Diplocladium* conidia and conidiophores produced by *Mycogone perniciosa* shows that in the still and moist air of a culture tube, the conidia can, on rare occasions, be found in a group of as many as six at the tip of a branch. Here they appear to be held together by moisture only. Costantin and Dufour (1, p. 463) have remarked: 'En prenant quelques précautions en faisant la préparation, on peut constater que les grandes spores sont réunies en petit nombre en capitale à l'extrémité des branches.' This statement refers to the 'Verticillium à grandes spores' which, on the authority of Costantin and Dufour (1, p. 469), produces *M. perniciosa*. The following words of these two authors (2) leave no doubt as to this: 'La coexistence des deux sortes de spores sur des filaments en continuité les uns avec les autres ne laisse aucun doute sur l'identité spécifique de ces deux formes.' In pure culture, again, they obtained the two forms of the fungus, i.e. the 'Verticillium à grandes spores' and the chlamydospores.

This fungus is distinct from the 'Verticillium à petites spores' which F. E. V. Smith (6) identifies as *Cephalosporium Constantinii*. Evidently, as Smith points out, (6, p. 82) Costantin and Dufour were concerned with two distinct diseases, viz. *M. perniciosa* (= 'Verticillium à grandes spores') on certain mushrooms, and *C. Constantinii* (= 'Verticillium à petites spores') on others. In only one instance were the two diseases found by Costantin and Dufour on one and the same mushroom.

<sup>4</sup> The average size of the *Diplocladium* spores of *M. perniciosa*, based on 1,100 measurements by the writer, is  $19.4\mu \times 4.6\mu$  and the variation  $8\mu - 40\mu \times 3\mu - 8\mu$ . The spores measured were derived from cultures of the fungus on coconut agar and from the fungus on sclerodermoid mushrooms. The diseased mushrooms (480 measurements) were from three different sources. Subcultures from one original isolation (420 measurements) and subcultures of American origin (200 measurements), kindly sent by Dr. E. B. Lambert, were used. Measurements of the American and English material agreed. Costantin and Dufour (1, 2) give  $8\mu - 20\mu \times 3\mu - 3.5\mu$  and Smith (6) gives  $15\mu - 20\mu \times 3\mu - 4\mu$  as the variation for the *Diplocladium* spores growing on mushrooms. Veihmeyer (8, p. 7) gives the average of the larger 2-celled spores as  $20\mu \times 3.5\mu$ .

the present fungus the main axis of the conidiophore is septate and the branches, borne in distinct whorls, are septate at the base. Both fungi produce spore-masses. The *Verticillium* here described forms occasionally



TEXT-FIG. 1.

TEXT-FIG. 2.

TEXT-FIG. 3.

TEXT-FIG. 1. Conidia of *Verticillium Malthousei* sp. nov. from mushroom. Camera lucida drawing.  $\times 1400$ .

TEXT-FIG. 2. Branching of conidiophores of *V. Malthousei*. (Semi-diagrammatic).

TEXT-FIG. 3. Conidiophore of *V. Malthousei*. (Semi-diagrammatic).

in culture simple conidiophores (Right of Text-fig. 2) as laterals from a hypha, but other laterals are always to be found which grow to a greater length and produce the typical whorls of branches (Text-fig. 2). On mushrooms, the conidiophores are always verticillately branched (Text-fig. 3), and it is presumed that Smith in his investigations with fresh mushroom material could not have failed to observe them had they been present. The writer has not met with *C. Costantinii* as a disease of mushrooms, and though there is some possibility that a fungus, accepted as a *Cephalosporium* in culture, may prove to be in reality a *Verticillium*, the identity of *C. Costantinii* with the fungus here described is not considered likely.

The present writer is unable to agree with Smith's determination that *C. Costantinii* is the same as the 'Verticillium à petites spores' of Costantin and Dufour (1). In their description, Costantin and Dufour ((1), p. 466) not only mention verticils but they figure the spore-bearing branches in whorls ((1), Pl. XIX, Figs. 43, 44, 45). The matter of this identification is reserved for future discussion when cultures of *C. Costantinii* have been examined.

The identity of a fungus associated with a disease of mushrooms in Vienna and which was incompletely described by Stapf (7) must remain uncertain. Stapf identified it as *Verticillium agaricinum* Corda, a conidial stage of *Hypomyces ochraceus* Pers., though he was unable to observe the ascomycetous form. He found a few *Mycogone* spores, once between the lamellae and once on a mycelial strand, but it seems clear from his account of cultures remaining white, without chlamydospore-formation,

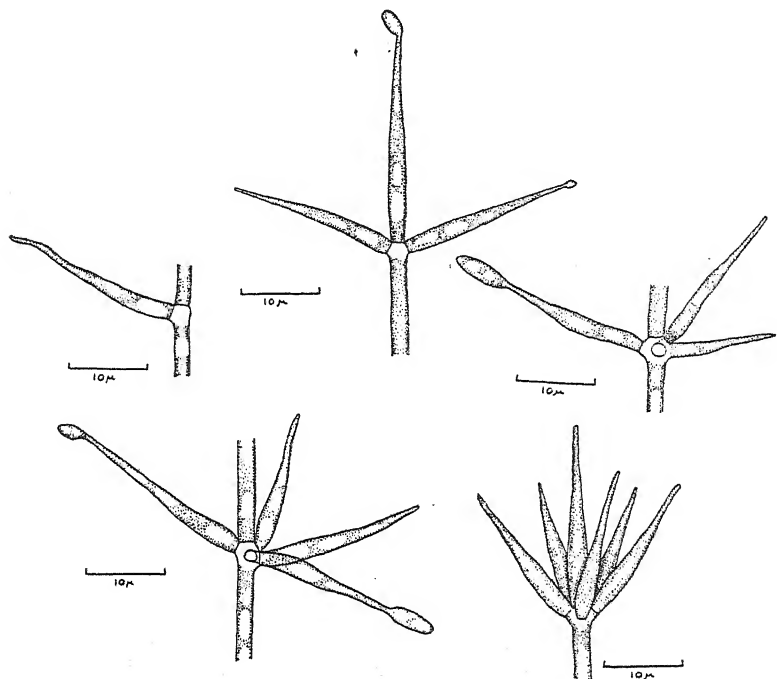
and of conidiophores bearing balls of spores, that he could not have been concerned with the so-called *Verticillium* stage of *M. perniciosa*. According to Veihmeyer ((8), p. 5), Magnus (4), in discussing the work of Stapf, was of the opinion that *M. rosea* Lk., rather than *M. perniciosa*, may have been concerned with the disease at Vienna, while Smith ((6), p. 81) assumes that the *V. agaricinum* of Stapf is to be considered as none other than *M. perniciosa*. Some of the disease symptoms recorded by Stapf agree with those caused by the *Verticillium* described in the present paper, e.g., the brown discoloured spots or patches on the upper surface of the pileus but, on the other hand, the symptoms of wet-rot with unpleasant smell, agree with those commonly induced by *M. perniciosa*. Owing, therefore, to the lack of sufficient descriptive details in the account by Stapf of his fungus, it must remain uncertain whether his *V. agaricinum* may or may not have been the *Verticillium* here described.

Distinction having been drawn between the fungus with which this paper is concerned and other fungi described by previous workers, one further comparison remains to be made. In 1901, G. T. Malthouse (5) recorded a mushroom disease in Edinburgh which was caused by a *Verticillium* to which he gave no specific name. The symptoms described and shown in the illustrations by Malthouse agree completely with those met with in the present investigation, notably in the splitting and downward peeling of the stipe and in the type of deformity. These are particularly well shown in his paper (5) in Pl. XIX, Fig. 4; Pl. XX, Fig. 8; and Pl. XXI, Fig. 10. The verticillate branching and aseptate spores drawn by Malthouse in Pl. XXII, Fig. 15, show that he was concerned with a true *Verticillium*. He states also ((5), p. 185) that no other stage of the fungus was formed in his cultures, and it may therefore be assumed that he was not working with the *Diplocladium* stage of *M. perniciosa*, for with that fungus chlamydospores would undoubtedly have been formed in all media. The branching of the *Verticillium* conidiophores described by Malthouse was in whorls, 2-7 in number on each conidiophore, and the 2-5 branches in the whorl each bore a single<sup>1</sup> spore. The conidia measured  $4\mu-7\mu \times 1.5\mu-2.75\mu$ , which agrees with the average dimensions of the conidia of the fungus now being considered. Malthouse's statements, however, that the parasitic hyphae are thick and with dense contents, and that they stain deeply with Bismarck Brown are not in agreement with observations by the writer. In the light of present knowledge as to the identity of the various fungi causing mushroom diseases, it is concluded that the *Verticillium* of Malthouse is the same as that described in the present paper, and on that account the name *V. Malthousei* sp. nov. is proposed.

*V. Malthousei* sp. nov. Mycelium white, thinly woolly, becoming

<sup>1</sup> In the fungus described in the present paper also, only one spore will be found at the tip of a conidiophore branch if it is examined in water.

greyish white on conidiophore-production. Hyphae creeping, septate, hyaline, branched,  $1-3\ \mu$  in diameter,  $4-5\ \mu$  in diameter where branches arise and  $3-7\ \mu$  in diameter, torulose and still hyaline, when in the resting



TEXT-FIG. 4. The mode of branching of conidiophores of *V. Malthousei* Camera lucida drawings.  $\times 1000$ .

condition in culture. Conidiophores lateral or terminal, upright, septate, sometimes simple,  $10-200\ \mu \times 1.5-2.0\ \mu$ , but generally verticillately branched and up to  $910\ \mu \times 1.5-5.0\ \mu$ . Secondary branches usually septate at base, rarely with an additional septum,  $20-40\ \mu \times 2-3\ \mu$ , tapering to  $1\ \mu$  at the tip, arising in whorls. Whorls 1-10 in number on main axis and composed of 2-12 branches which are usually simple, rarely with secondary whorls. Conidia oblong or cylindrical, occasionally irregularly fusoid, ends obtuse, hyaline, unicellular,  $3-16\ \mu \times 1.5-5.0\ \mu$ , average  $6.6\ \mu \times 2.5\ \mu$ .<sup>1</sup> Conidia are cut off singly at the tip of the branch but remain clustered in spore-masses with mucilage. Spore-masses globular or nearly so,  $4-14\ \mu$  in diameter, swelling and dissociating when wet, containing up to 64 conidia or more.

Parasitic on cultivated mushrooms.

*V. Malthousei* sp. nov. Mycelium album, tenuiter lanatum, ob conidiophora numerosissima demum griseo-albescens. Hyphae repentes, septatae, hyalinae, ramosae,  $1-3\ \mu$  diametro, sub ramis  $4-5\ \mu$  diametro, in vitro

<sup>1</sup> 1200 conidia were measured.

interdum torulosae sed semper hyalinae, 3-7  $\mu$  diametro. Conidiophora lateralialia vel terminalia, erecta, septata, interdum simplicia 10-200  $\mu \times$  1.5-2.0  $\mu$ , plerumque ramulis verticillatis praedita, ad 910  $\mu \times$  1.5-5.0  $\mu$ . Ramuli basi septo plerumque delimitati, rarissime iterum medio uniseptati, 20-40  $\mu$  longi, 2-3  $\mu$  diametro, apicem versus ad 1  $\mu$  diametro angustati. Verticilli 1-10, e ramulis 2-12, plerumque simplicibus, rarius verticillis secundariis praeditis compositi. Conidia oblonga vel cylindrica, interdum irregulariter fusiformia, apicibus obtusis, 3-16  $\mu \times$  1.5-5.0  $\mu$ , longitudine media 6.6  $\mu$ , latitudine media 2.5  $\mu$ . Conidia singulatim e conidiophori apice abstricta, sed in capitula mucilagine cohaerentia. Capitula globosa vel subglobosa, 4-14  $\mu$  diametro, madentia expansa atque diffuentia, conidia ad 64 vel ultra includentia.

Habitat in Psalliota campestre culta.

#### TECHNIQUE OF EXAMINATION.

Spores and conidiophores are readily obtained from the surface of diseased mushrooms, and may be examined in water or in iodine solution with the twelfth-inch objective. Using a lower power, identification of the fungus can be made by cutting deformed mushrooms longitudinally and examining with the help of overhead illumination the cavity in the stipe, the split mushroom being held on a slide without the use of any liquid. With tube cultures the production of simple conidiophores, at a distance of about 1 mm. behind the fringe of advancing hyphal tips, can be observed. They arise as aerial lateral branches (Text-fig. 2) of the hyphae, while further back, in the older parts of the culture, they are longer and bear whorls of branches (Text-fig. 2). The spore-masses dissociate on contact with water, and it is therefore necessary to measure them in the dry condition; this is done either through the glass of a culture tube or by examination of the surface of plate cultures or of diseased mushroom tissue. The production of spore-masses at the tips of the conidiophores, accessible for microscopical examination, may be secured by floating spores on the surface of water in a watch-glass; the spores germinate with a germ tube from one or both ends and conidiophores soon grow from the hyphae which are formed.

In old cultures the verticillate conidiophores are not always apparent, the culture consisting of torulose hyphae below the agar and of white mycelium on the surface. Conidiophores are, however, produced after three or four days when thin slices of agar are cut from such cultures and are floated on water. By another method, larger blocks of agar are cut out and are placed on glass slides in a moist atmosphere; conidiophores are produced on the freshly-cut surfaces of the agar.

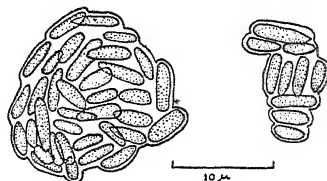
Detailed examination of the spore-masses, e.g. to obtain measure-



ments or to count the spores held in the mass, is made possible by placing a small piece of agar culture, or of mushroom tissue on a slide and laying on this a dry cover-glass. The cover-glass settles down gradually and no



TEXT-FIG. 5.



TEXT-FIG. 6.

TEXT-FIG. 5. The formation of the spore-masses of *V. Malthousei* at the tips of the secondary branches. Abstricted spores are shown adhering to those which are still developing.

TEXT-FIG. 6. Large and small spore-masses of *V. Malthousei* observed in process of dissociation. From agar culture. The conidia are commonly smaller in culture than when grown on mushroom. Camera lucida drawing.  $\times 1300$ .

pressure is applied to it. The spore-masses can be measured with the sixth-inch objective; those in contact with the cover-glass dissociate only slowly because of the limited amount of moisture condensing on the glass. Using the same method and with the twelfth-inch objective, the mode of formation of the mass (Text-fig. 5) can be observed, and the spores contained in the larger masses can be counted (Text-fig. 6). A gradual hardening of the mucilage of the spore-masses probably occurs, for if conidiophores are removed from the surface of old dry cultures on manure compost and are placed in water, the masses part in a whole condition from the tips of the branches, and separation of the spores is extremely slow. By this method also counts can be made.

The tenacity and tensility of the mucilage of the spore-masses are shown by its power in holding insects such as springtails; the tenacity is also observable if conidiophore-bearing fragments of agar or of mushroom tissue are examined, without a cover-glass, under the microscope. The conidiophores exposed to the air twist around on their long axis and some of the spore-masses, borne on different conidiophores, come into contact; they fuse immediately and the conidiophores become locked together. The effect can be exaggerated by creating a slight draught on one side of the microscope stage.

#### SUMMARY.

1. A disease of cultivated mushrooms, noted in October, 1929, and with which *Verticillium* sp. was associated, is described. The most important symptoms are: (a) deformity of the entire mushroom which is covered with a white, or greyish-white, close mycelium; (b) the production of greyish-white spots on the pileus of mushrooms not deformed; or (c) the occurrence of white infected areas on stipe or gills.

2. The fungus was isolated and single-spore cultures obtained.
3. An experiment on the thermal death-point showed that in a temperature of 40° C. the fungus in agar culture is killed by exposure for six hours or more.
4. Different methods of inoculation were tested.
5. Infection experiments, which proved the parasitism of the fungus, confirmed the suspicion that only inoculation at the earliest stage of growth causes the production of deformed mushrooms, while inoculation at later stages results in local infections such as the typical spotting of the pilei. Control measures are suggested.
6. The fungus is compared with others known to be parasitic on mushrooms. It is considered identical with one which was incompletely described by G. T. Malthouse in 1901.
7. The name *V. Malthousei* sp. nov. is proposed.
8. The technique adopted for microscopical examination of the conidiophores and spore-masses is described.

The writer is indebted to Dr. G. H. Pethybridge and Mr. W. C. Moore for bringing to his notice the paper (5), in which a *Verticillium* disease of mushrooms was recorded in 1901, and to Miss E. M. Wakefield for assistance in writing the description of *V. Malthousei*.

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## EXPLANATION OF PLATES XXVI AND XXVII.

Illustrating Mr. W. M. Ware's paper on 'A Disease of Cultivated Mushrooms Caused by *Verticillium Malthousei* sp. nov.'

## PLATE XXVI.

Fig. 1. A common type of naturally-occurring deformity of mushrooms caused by *V. Malthousei*. The white covering on the surface consists of conidiophores of the parasite. Numerous springtail (seen as black dots) are held fast by the mucilage of the spore-masses. Nov. 14, 1929. Natural size.

Fig. 2. A group of four mushrooms (brown variety) united at the base. Naturally-occurring deformity, due to *V. Malthousei*, is seen in only one of the four and on this numerous springtails are held by the mucilage of the spore-masses. November 14, 1929.  $1\frac{1}{2} \times$  Natural size.

Fig. 3. Types of deformity resulting from early inoculation with spore-suspensions of *V. Malthousei*. The downward peeling of the stipe frequently but not invariably occurs. Inoculated April 8, photographed May 13, 1930. Natural size.

Fig. 4. Mushrooms in Box 3 (see p. 775) of which the right half was atomized with a spore-suspension of *V. Malthousei* and the left half with water only. The young mushrooms were already protruding from the casing soil when the atomization was done on July 5; the result of this late-stage inoculation is the grey spotting of the pilei. Photographed July 10, 1930. One-third natural size.

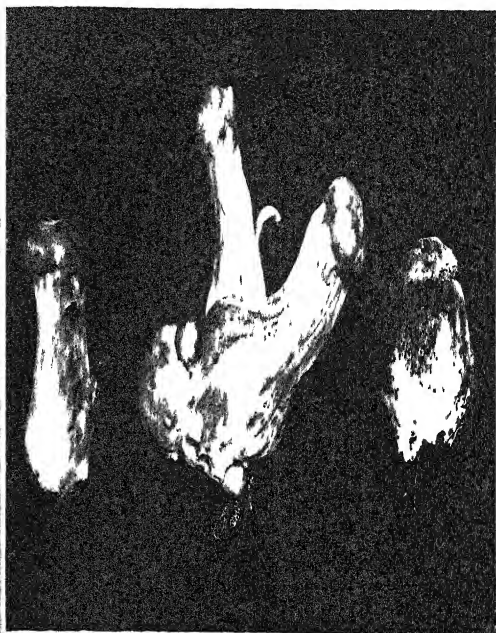
## PLATE XXVII.

Fig. 5. Cut mushrooms inoculated April 2, 1932 by drawing radially along the gills a camel-hair brush moistened with a spore-suspension of *V. Malthousei*. Upward spread of the fungus on one side of the cut stipe is shown. Temp. 18-20° C. Photographed April 5, 1932. Four-fifths natural size.

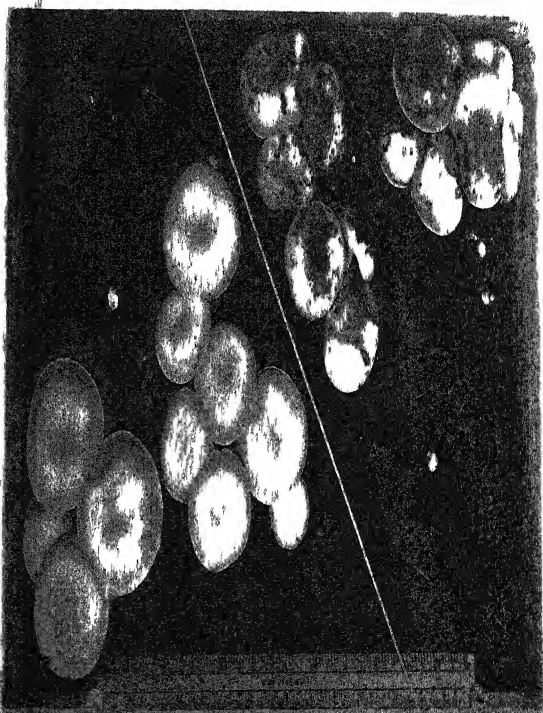
Fig. 6. A mixed culture of *V. Malthousei* and *Mycogone perniciosa* on coconut agar after twenty-one days' growth at 13° C. No repulsion occurs. Cultures of *V. Malthousei*, after further growth, become completely white with increased surface mycelium such as is shown at the centre of the culture illustrated. The characteristic white fringe, bearing the *Diplocladium* stage, is clearly shown in the culture of *M. perniciosa*. Four-fifths natural size.



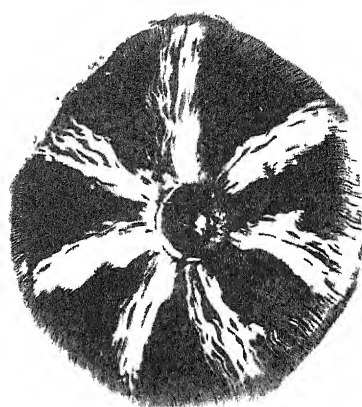
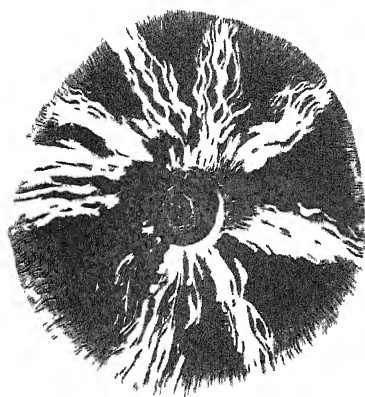
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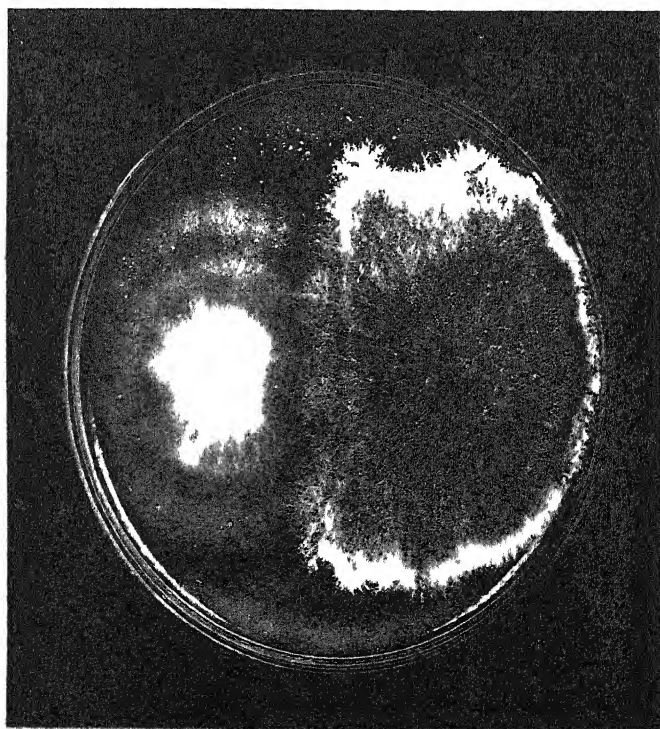
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6



# A Desert Protosiphon, *Protosiphon botryoides* (Kütz) Klebs, var. *deserti*.

BY

A. A. NAYAL, M.A. (Camb.).

(*Department of Botany, Egyptian University, Cairo.*)

With seventeen Figures in the Text.

## CONTENTS.

	PAGE
I. INTRODUCTION . . . . .	787
Systematic position . . . . .	788
II. GENERAL MORPHOLOGY . . . . .	789
III. LIFE-HISTORY . . . . .	790
Production of swarmers . . . . .	790
Structure and behaviour of swarmers . . . . .	790
Development of zoospore . . . . .	790
Fusion of gametes . . . . .	791
Development of zygote . . . . .	791
Vegetative reproduction . . . . .	791
Resting spores . . . . .	792
General remarks on the life-history and dispersal . . . . .	792
Summary of life-history . . . . .	793
IV. ENDURANCE OF DIFFERENT TEMPERATURES BY <i>P. BOTRYOIDES</i> . . . . .	793
1. Actual Egyptian Records of Temperature of Various Surfaces . . . . .	793
2. Effect of High Temperature on Protosiphon Cysts . . . . .	794
3. Effect of Low Temperature on Protosiphon Cysts . . . . .	795
4. Effect of Different Temperatures on the Germination of Protosiphon Cysts . . . . .	796
5. Summary of Temperature Experiments . . . . .	796
V. BEHAVIOUR OF PROTOSIPHON IN THE PRESENCE OF COMMON SALT . . . . .	796
VI. CONCLUSION . . . . .	797
LITERATURE CITED . . . . .	798

## I. INTRODUCTION.

THE green alga *Protosiphon botryoides* (Kütz) Klebs var. *deserti* occurs both in the Nile Valley and in certain localities in the desert to the east of Cairo.

On November 12, 1930 there was a very heavy shower of rain which  
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brought down torrents of water loaded with silt from the desert near the Mokattum Hills east of Cairo. The run off or 'Seyl' water, following the track of the desert railway line, accumulated in low-lying areas. Thus the grounds of the Faculty of Science at Abbassia received a generous covering of this silt. As the water evaporated the silt consolidated to a layer of colloidal mud 2 or 3 in. thick, and in due course as it dried further the silt began to crack in characteristic patterns, eventually setting into a hard layer like earthenware which flaked off horizontally. By the time the first fissuring occurred, a succession of plants began to appear at the surface. First came *Protosiphon botryoides* (Kütz) Klebs var. *deserti* followed by *Botrydium granulatum* (L.) Grev., *Riccia*, Moss protonema, several forms of fungi, and finally the seedlings of certain flowering plants which occur in the desert.

On October 1, 1931 there was a similar shower and silt was again brought down by Seyl water to the Faculty grounds. As the water dried up the silt became covered with a green carpet of Protosiphon, much more abundant than in the previous year, whereas *Botrydium*, *Riccia*, and Moss protonema appeared rather late and were much less common than before.

In a fortnight's time the green coloration turned red in the areas exposed to the sun, whilst in the shady places, where the silt remained damp for a longer period, the green colour was prolonged. This change of colour was due to the appearance of the cysts of Protosiphon.

#### *Systematic position.*

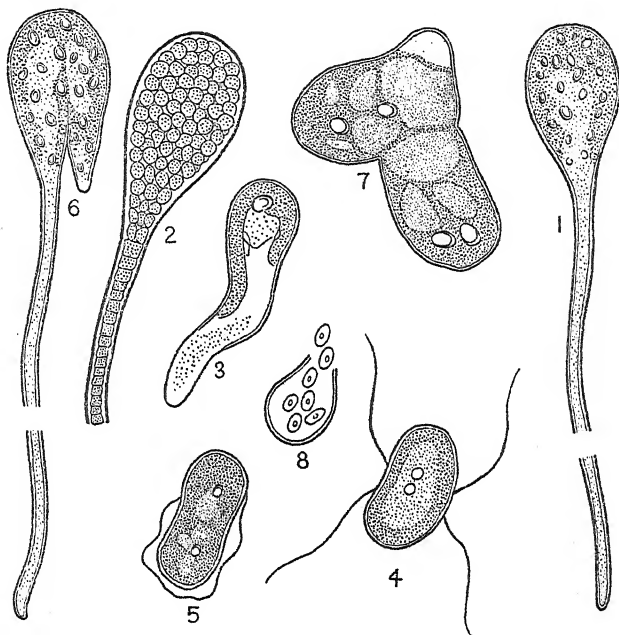
Prior to Klebs's (1) investigations Protosiphon had been confused with *Botrydium* with which it generally occurs in nature. Klebs was the first to point out clearly that Rostafinski and Woronin (3) in their description of *Botrydium* had combined two distinct Algae, superficially similar but unrelated. *Botrydium* was subsequently placed under the Heterosiphonales, whilst Protosiphon is considered to be a member of the Chlorococcales and the only known representative of its family the Protosiphonaceae. There is only a single species known, *P. botryoides* (Kütz) Klebs. In the present investigation it was found that the characters of our plant and its behaviour, especially under different temperatures, show marked differences from those of *P. botryoides* (Kütz) Klebs as described by Klebs; but having had no opportunity of examining that species it seems premature to establish a second one; nevertheless I feel justified in proposing, at least, a new variety.

*Protosiphon botryoides* (Kütz) Klebs Mature plant (maximum) 1.2–1.4 mm. l., 0.5 mm. br.; Gametes normally fuse sideways.

*Protosiphon botryoides* (Kütz) Klebs var., *deserti*. Mature plant (average) 1.25–1.6 mm. l., 0.13–0.18 mm. br.; (maximum) 1.95 mm. l., 0.3 mm. br.; Gametes normally fuse end-to-end.

## II. GENERAL MORPHOLOGY.

*Protosiphon botryoides* (Kütz) Klebs var. *deserti* usually occurs in clusters on the surface of the soil. With the help of a hand-lens the clusters appear as small, greenish, swollen vesicles closely packed together and firmly rooted in the soil.



FIGS. 1-8. Fig. 1, whole plant,  $\times 80$ . Fig. 2, cyst formation,  $\times 80$ . Fig. 3, germination of zoospore with commencing rhizoid,  $\times 600$ . Fig. 4, fusion of gametes; the two nuclei before fusion,  $\times 1000$ . Fig. 5, germination of zygote,  $\times 1000$ . Fig. 6, longitudinal division of mature specimen,  $\times 80$ . Fig. 7, vegetative multiplication (budding),  $\times 400$ . Fig. 8, escape of non-motile spores,  $\times 700$ .

The plant (1.25-1.6 mm. long) is unicellular consisting of two regions, a green aerial portion and a colourless subterranean rhizoid (Fig. 1). The green portion (0.13-0.16 mm. br.) is generally elongated and contains a single parietal reticulate chloroplast with many pyrenoids imbedded in it. The subterranean portion (3-5 times the length of the aerial portion) is tubular and rarely branching. The cytoplasm forms a lining layer to the wall and contains many small scattered nuclei internal to the chloroplast (Fig. 14). There is a central vacuole continuous throughout.

Under dry conditions the cytoplasm breaks up into a large number of cysts which have thick walls and red contents. In the rhizoidal part the cysts form one or sometimes two longitudinal rows (Fig. 2). The cysts (20-30  $\mu$  br.) are multinucleate and have a spherical or angular form due to mutual pressure.

III. LIFE-HISTORY.<sup>1</sup>*Production of swarmers.*

When cysts are submerged in water for several hours at ordinary room temperatures, they produce swarming spores. The contents of the cyst divide up into a number of units which separate off, acquire cilia, and move about inside the parent wall. Their movement becomes more and more active and ultimately one emerges and escapes through the wall of the cyst and that of the parent plant, while the others follow in succession.

*Structure and behaviour of swarmers.*

Swarmers are normally biciliate and phototactic. They come out red in colour, but they gradually turn green except for the red eye spot which is situated at one side near the base of the cilia. The cilia are about twice the length of the swarmer. The chloroplast occupies the peripheral layer of the cytoplasm except at the base of the cilia, which is clear and contains two contractile vacuoles. There is generally a single nucleus in each spore.

Swarmers vary in form. They may be rounded or elongated (Figs. 9-13). The rounded swarmers (Fig. 9) ( $5-7.5\ \mu$  in diam.) behave as zoospores; germinating directly. The elongated spores (Fig. 10) ( $7.5-9\ \mu \times 3.4\ \mu$ ) are facultative gametes. They may fuse together in pairs or behave as ordinary zoospores.

Sometimes masses of two or more swarmers (Figs. 11-13) which have not separated off from each other, escape from the germinating cysts. Each mass has two or more differently oriented pairs of cilia. Some of them separate off into their units while swarming (Fig. 12) whilst others behave as single zoospores.

*Development of zoospore.*

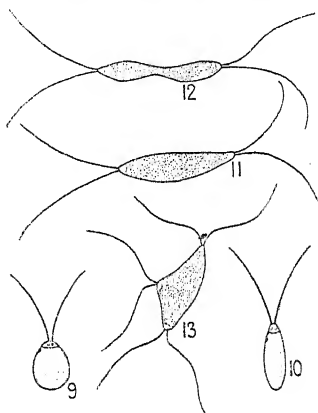
The zoospores move about actively for a short period; they then come to rest, round themselves up, lose their cilia, and acquire cell-walls (Fig. 15). As the spore enlarges the chloroplast grows and becomes reticulated. There is at first a small, more or less central pyrenoid which enlarges and divides. The daughter pyrenoids separate off, multiply and scatter themselves in the chloroplast. The nucleus also divides and multiplies. One end of the spore elongates to form the colourless tubular part, while the other end enlarges to form the green aerial portion of the plant (Fig. 3). When the rhizoid comes into contact with a small particle it curves round it, but when the plant is grown on damp mud the rhizoid penetrates it vertically.

<sup>1</sup> The study of the life-history was based upon observations on cultivations on silt and on ordinary garden soil, supplemented by synthetic cultures (Knop's solution and Knop's agar).

*Fusion of gametes.*

Gametes are isogamous. Those coming out from the same mother cyst do not fuse together even when put under activating conditions for copulation.

Gametes swim about like the zoospores, but when two physiologically different gametes meet, they entangle each other with their cilia, come into contact by their anterior ends and move about together for a short time; they then become quiescent and begin to fuse (Fig. 4). The point of contact dissolves, the opening widens, the corresponding parts of the two gametes unite and the four cilia disappear. The two nuclei approach each other and meet in the middle. The resultant body soon rounds off and forms a lobed thick-walled zygospore (Fig. 16). The number of lobes varies from 5-8 but mostly there are only 6.



FIGS. 9-13. Swimmers. Fig. 9, zoospore. Fig. 10, facultative gamete. Figs. 11, 12, and 13 compound swimmers. Fig. 12, shows division into two units. All  $\times 1150$ .

Gametes are physiologically different. Two gametes may entangle each other with their cilia and remain so for a while, but they soon free themselves off and swim about actively. This process may be repeated more than once and it is only when two appropriate gametes come into contact that actual fusion takes place. Gametes that fail to fuse may behave as zoospores.

*Development of zygote.*

Newly formed zygotes germinate directly when transferred to new cultures and properly illuminated. Old zygotes require a period of rest before germination. When exposed to dry conditions they become red in colour. On germination the zygote elongates, bursts through its thick wall at one end and grows directly into a new plant (Fig. 5).

*Vegetative reproduction.*

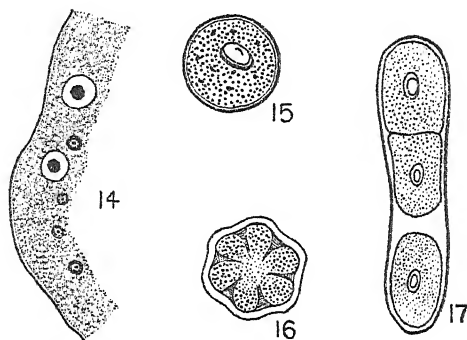
Vegetative multiplication takes place by longitudinal division of the green portion into two parts. Division starts from the lower part, close by the origin of the rhizoid, and progresses towards the apex (Fig. 6). It may not be complete and the two halves remain attached to each other and form cysts. If favourable conditions are prolonged, the two halves separate off, one of them remains attached to the main rhizoid, whilst the other elongates to form a new rhizoid for itself.

Plants grown in cultures vegetate freely. Young plants may produce one or more lateral projections (buds) that grow out, separate off, and

produce new plants (Fig. 7). Various formed few-celled colonies are commonly seen in cultures.

### *Resting spores.*

Under unfavourable conditions resting spores may be formed at any stage. In young plants the cytoplasm breaks up into two or more non-



FIGS. 14-17. Fig. 14, part of section of the aerial portion showing pyrenoids and nuclei,  $\times 1470$ . Fig. 15, zoospore come to rest,  $\times 1330$ . Fig. 16, zygote,  $\times 1200$ . Fig. 17, formation of resting spores,  $\times 530$ .

motile spores (Fig. 17). Spores just germinating may cease to grow and enter on a resting stage. Under dry conditions resting spores become red in colour.

Resting spores, transferred to new cultures produce swarmers, but small ones may germinate directly. Swarmers produced from red spores come out red in colour, those from green spores come out green.

Sometimes the production of swarmers is completely suppressed. The contents of the resting spore divide up into a number of units, which grow in size inside the parent wall, then burst through and emerge as non-motile spores (Fig. 8).

### *General remark on the life-history and dispersal.*

Environmental conditions play an important part in the life-history of the plant. Moderate temperature activates its growth. Moist conditions prolong its vegetative phase. Sufficient food supply encourages vegetative multiplication. Light activates the copulation capacity of the swarmers and also growth in all stages. On the advent of adverse conditions resting spores are formed and on drought they become red in colour. Resting spores, on germination, produce swarmers or non-motile spores, but they may grow directly into new plants. The life-cycle may be completed in 12 or 15 days, or it may be prolonged up to 40 days or even more.

The chief distributing agent is the Seyl water which carries the cysts from the desert to the Nile valley.

*Summary of Life-history.*

*Protosiphon botryoides* (Kütz) Klebs var. *deserti* is unicellular and coenocytic. It has a green aerial portion and a colourless tubular rhizoid. On drought the plant forms red coloured cysts. Cysts submerged in water produce biciliate swimmers which are either zoospores or facultative gametes. Zoospores germinate directly. Facultative gametes may fuse together in pairs end to end, producing thick-walled stellate zygospores. Gametes that fail to fuse behave as zoospores. The zygospore on germination gives rise to a new plant. The plant vegetates freely under favourable conditions. Under adverse conditions resting spores are formed at any stage. On drought they become red in colour. When wetted they may produce swimmers, or non-motile spores, or grow directly to new plants.

#### IV. ENDURANCE OF DIFFERENT TEMPERATURES BY *P. BOTRYOIDES* (KÜTZ) KLEBS VAR. *DESERTI*.

In view of the different temperatures to which the surface soils of the desert are subjected, it appeared desirable to expose the resting stages of *Protosiphon* to analogous temperatures for varying periods, to ascertain whether our plant is capable of enduring them.

The records taken at Wadi Digla show the actual temperatures of the sand in August, a hot time of the year, in March, a cool dry time, and in December, a cool moist time of the year. Wadi Digla is a narrow ravine in the desert running through a limestone plateau to the east of the Nile just south of Cairo. The wadi (valley) is about two hundred feet deep and is a dry water-course through which water occasionally flows after rain.

The temperature indicated by the black bulb thermometer may be regarded as a measure of the amount of radiant heat reaching the surface layer of the soil. The character of the surface has a considerable influence upon the soil temperatures. In the sand the maximum is lower than that in the soil. The soil has a greater absorbing power and little of the radiant heat is lost by reflection.

##### 1. *Actual Egyptian Records of Temperature of Various Surfaces.*

Surface layer of sand at Wadi Digla.	August, 1922.	March, 1923.	December, 1923.
	° C.	° C.	° C.
Absolute maximum temp.	58.2	43.6	29.3
Mean maximum temp.	56.1	39.2	28.5
Absolute minimum temp.	17.5	5.7	6.6
Mean minimum temp.	20.6	7.6	8.4

(Technical and Scientific Service. Bulletin no. 50, Ministry of Agriculture Cairo.)

	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
1. Highest black bulb recorded at Assuan 1910-30	62	68	72	78	80	81	80	81	79	74	69	60
2. Highest black bulb recorded at Giza 1928-31	60	64	67	79	81	75	71	73	73	72	64	60
3. Highest temperature of surface of roof of Physical Dept. <sup>1</sup> , Cairo, 1927	30	35	44	46	62	54	55	60	51	50	40	32
4. Mean temperature of (3) 1927	26	27	37	42	51	52	53	53	46	43	33	29
5. Mean air temperature taken at the same time with (4) 1927	19	18	23	27	33	34	35	34	31	31	26	22

(These records were obtained from the Physical Department, Cairo.)

On the whole it appears safe to say that the sand-surface temperature never reaches 80° C. in Egypt, and probably does not exceed 70° C.

#### 2. *Effect of High Temperature on Protosiphon Cysts.*

Pieces of silt covered with Protosiphon cysts were placed in ovens having the undermentioned temperatures. Parts of these were taken out of the ovens after the mentioned times and submerged in distilled water in glass jars. The following results were obtained :

Temp. of oven °C.	Time in hours or days.	Observations.
30	1 day, 2 days, and so on for 60 days	Swarms appeared in abundance the next morning after submergence in water.
37	1 day, 2 days, and so on for 48 days	Same as before.
55-60 <sup>2</sup>	1 day, 2 days, and so on for 5 days	Same as before.
"	9-12 days	Production of swarms continued for 3 successive days after submergence in water. A few cysts only did not germinate.
"	15-16 days	Production of swarms continued for 4 successive days after submergence in water. Many cysts did not germinate.
"	22 days	Production of swarms continued for 9 successive days after submergence in water. Many cysts did not germinate.
70	24 hours, 48 hours, and 72 hours.	Swarms appeared in abundance the next morning after submergence in water.

<sup>1</sup> Dirty limestone with some dust.

<sup>2</sup> The temperature of the oven fluctuated from 55° C.-60° C. during the experiments.

Temp. of oven °C.	Time in hours or days.	Observations.
70	4-7 days	Production of swarmers continued for few successive days after submergence in water.
„	8-13 days	Very few swarmers were produced for a few successive days after submergence in water. Many cysts did not germinate.
„	16 days	A very small number of swarmers appeared after several days submergence in water. Many cysts did not germinate.
78-80 <sup>1</sup>	18 hours.	Few swarmers were produced for few successive days after submergence in water. Many cysts did not germinate.
80-83	16 hours	Very few swarmers were produced after two days submergence in water.
73-81	4 hours	The temperature of the oven rose gradually from 73° C. to 81° C. in 3 hours and remained at 81° C. for 1 hour. Swarmers appeared in abundance the next morning after submergence in water.
75-86	17½ hours	The temperature remained nearly constant at 75° C. for 17 hours, then rose gradually up to 86° C. and remained at 86° C. for 10 minutes. Swarmers appeared in abundance the next morning after submergence in water.
75-91	18 hours	The temperature was exactly as before, but after the 17½ hours it rose to 91° C. remaining 10 minutes. Swarmers appeared in abundance the next morning after submergence in water.

N.B.—Very few zygotes were formed in most cases. At the higher limits hardly any zygotes formed.

### 3. Effect of Low Temperature on *Protosiphon* Cysts.

Experiments similar to the previous high temperature ones were performed, pieces of silt covered with cysts being placed in cold chambers. The following table shows the result.

Average Temp. °C.	Time in days.	Observations.
5 <sup>2</sup>	1 day, 2 days, and so on for 5 days	Swarmers appeared in abundance the next morning after submergence in water. Many zygotes were formed.
„	6-12 days	Production of swarmers continued for few successive days after submergence in water. Few zygotes were formed. Some cysts did not germinate.
0	1 day, 2 days, and 3 days	Swarmers appeared in abundance the next morning after submergence in water. Many zygotes were formed.
„	4-10 days	Production of swarmers continued for few successive days after submergence in water. Very few zygotes were formed. Many cysts did not germinate.

<sup>1</sup> The temperature of the oven fluctuated from 78-80° C. during the experiments.

<sup>2</sup> Temperature of the cold chamber fluctuated 2° C. or 3° C. above and below the average temperature in all the above experiments.



#### 4. *Effect of Different Temperatures on the Germination of Protosiphon Cysts.*

As germination of cysts takes place in water it appeared desirable to find out the limits of water temperatures under which cysts can germinate.

Cysts, submerged in distilled water in glass jars, were placed in incubators having different temperatures and examined for germination. Swarmers were normally produced at different temperatures between  $12^{\circ}\text{C.}$ – $35^{\circ}\text{C.}$  Beyond these limits production of swarmers diminishes gradually as the temperature falls below  $12^{\circ}\text{C.}$  or rises above  $35^{\circ}\text{C.}$ , and at about  $3^{\circ}\text{C.}$  and  $57^{\circ}\text{C.}$  respectively, very few swarmers were produced.

#### 5. *Summary of Temperature Experiments.*

1. Cysts withstand different temperatures, from  $7$ – $50^{\circ}\text{C.}$ , for long periods with little effect and germinate directly when submerged in water.

2. They can endure  $50$ – $60^{\circ}\text{C.}$  for 15–8 days, respectively. If they are exposed to such temperatures for longer periods, 9–22 days under  $60^{\circ}\text{C.}$  and more than that under  $50^{\circ}\text{C.}$ , they undergo a period of rest varying from 2–9 days or perhaps more.

3. When exposed to  $70$ – $75^{\circ}\text{C.}$  their endurance becomes more limited, less than 3 days.

4. They withstand  $75$ – $83^{\circ}\text{C.}$  for only few hours.

5. When subjected to gradually rising temperatures many cysts withstood exposure to  $91^{\circ}\text{C.}$  for 10 minutes or perhaps more, with little effect.

6. Fusion of swarmers is more induced when cysts producing them were previously subjected to lower than to higher temperatures, and above  $60^{\circ}\text{C.}$  hardly any copulation takes place.

7. Cysts exposed to low temperatures,  $0$ – $3^{\circ}\text{C.}$  for more than 4–6 days respectively, produce swarmers that show a diminished capacity for copulation.

8. Germination of cysts takes place normally under different temperatures between  $12^{\circ}\text{C.}$ – $35^{\circ}\text{C.}$  Beyond these limits germination is reduced, and at about  $3^{\circ}\text{C.}$  and  $57^{\circ}\text{C.}$ , respectively, only few swarmers are produced.

#### V. BEHAVIOUR OF PROTOSIPHON IN THE PRESENCE OF COMMON SALT.

The silt brought down by the Seyl water was found to contain sodium chloride of the order of 0.1 per cent. In the Egyptian desert sodium chloride is sometimes present in a higher percentage. It was, then, found desirable to study the behaviour of the plant in the presence of this salt.

A piece of salt crust, taken from the Mariut district (dry marsh) containing 76.5 per cent. sodium chloride, was dissolved in different concentra-

tions in distilled water, and *Protosiphon* cysts were submerged in them. The following results were obtained :

% concentration of salt.	Germination.	After germination.
0.1-1	Normal germination. Few zygotes formed.	Normal growth.
1.2-1.4	Few swarmers were produced for several days after 3 days submergence. No zygotes formed.	Growth retarded.
1.6	Production of swarmers was less than before, and took place after 4 days submergence. Some cysts did not germinate.	Growth is very slow.
1.8-2.0	Only very few swarmers were produced after 5 days submergence. Many cysts did not germinate.	
2.2	Practically no germination took place.	

#### *Summary of Common Salt Experiments.*

1. Normal germination and growth takes place in different concentrations of common salt solutions up to 1 per cent.
2. As concentration goes higher, germination and growth are more reduced, and in 2 per cent. solution only few swarmers are produced and many cysts do not germinate at all.
3. Above 2 per cent. practically no germination takes place.

#### CONCLUSION.

The cysts of *Protosiphon botryoides* (Kütz) Klebs var. *deserti* can withstand high and low temperatures to a considerable extent. They appear to be quite suited to the dry conditions of the Egyptian desert. The surface temperature of the desert varies from a few degrees below 0° C., on some nights, to 60° C. or perhaps more on some summer days. It has already been mentioned that cysts exposed to 70° C. for several days continuously are not much affected, the majority of them germinating directly when submerged in water. When they are exposed to gradually rising temperatures many of them can endure higher degrees up to 91° C. with little impairment, but they cannot withstand temperatures above 78° C. when transferred *direct* to such higher temperatures. Cysts exposed to high temperatures for a lengthened period do not germinate directly, but require a period of rest before germination.

The desert receives a few showers of rain during winter, but in some seasons rain falls in early autumn or late spring. During the long summer period no rain falls on the desert. After a shower of rain in early autumn or late spring when the weather is rather warm, the surface layer of the desert dries up quickly, but when rain falls in winter the surface layer retains its moisture for a much longer period. The life-history of the plant seems fitted to withstand such vicissitudes. Warm weather stimulates

its growth and shortens its life-history. Moist, cool conditions prolong its vegetative phase. On the advent of dry conditions resting spores are formed at any time. If rain comes soon they germinate and grow, but if the dry condition is prolonged they turn red in colour and rest. Zygospores behave similarly. They may germinate directly or turn red in colour and rest. Under favourable conditions the plant vegetates freely so that its life-history is prolonged.

Germination of cysts takes place normally at different temperatures between 12° C. and 35° C. Beyond these limits germination takes place, but to a limited extent. On the whole it appears safe to say that the temperature of a mass of water in Egypt does not exceed these limits for any length of time, except in very severe seasons, under which conditions some cysts are not affected.

The percentage of common salt in the desert is very low, and in most places it does not exceed 0.2 per cent. As has already been mentioned cysts germinate quite normally and young plants grow well in different concentrations of common salt up to 1.0 per cent. Above such concentrations cysts may germinate, but only after a period of rest.

On the whole the plant shows a marked plasticity in the fact that it can exist under a very wide range of conditions and responds quickly and remarkably to any change in its environment.

It is a pleasure to take this opportunity to express my gratitude to Professor F. W. Oliver under whose guidance this work was done, for his unflinching assistance and suggestions. I wish also to express my thanks to Dr. H. E. Hurst, Director of the Physical Department, Cairo, for supplying me with Egyptian records of temperature and to members of the Botany Department of the Egyptian University who assisted me during this work.

All the figures illustrating this paper were drawn under the camera lucida, and from fresh material, except Figs. 9-31 and Fig. 14, which were fixed and stained.

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# The Development of *Entomophthora sphaerosperma* upon *Rhopobota vacciniana*.<sup>1</sup>

BY

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With Plates XXVIII and XXIX and one Figure in the Text.

## INTRODUCTION.

*ENTOMOPHTHORA sphaerosperma* has been one of the better known entomogenous fungi since Brefeld (1, 2, 3), beginning in 1870, published his series of papers dealing with its parasitism on *Pieris brassicae*, investigations that rivalled in importance the pioneer work already accomplished by Cohn (4) on the related form, *Empusa muscae*. Later studies have furthered the interest in this species by disclosing its wide distribution on a large variety of insects.

A new host was added in 1923 when, in Massachusetts, U.S.A., the writer found the fungus parasitic on the larvae of *Rhopobota vacciniana*; these small lepidopterous larvae feed on the foliage of the cultivated cranberry, *Vaccinium macrocarpon*, and often effect such serious ravages that only the brown skeletons of the leaves remain. It is from this fire-swept appearance of the cranberry vines, and from the shiny black cuticle of its own cephalic segments, that the insect larva derives its common name of 'black-headed fireworm'. This host is either the same species as, or very closely related to, *R. naevana*, a European insect whose larvae feed upon holly.

In the summer of 1927 the writer succeeded in transferring *E. sphaerosperma* from its insect host to pure culture upon non-living materials; the methods and media employed have been described in a previous paper (5). The fungus in artificial culture offered incomparable opportunities for controlled studies of infection and development. These studies have been made with particular attention to the relation between the parasite and its host, and the results are presented in this paper.

<sup>1</sup> Contribution from the Laboratories of Cryptogamic Botany, Harvard University, no. 112.

## METHODS.

Large numbers of larvae were collected by sweeping the tips of badly infested cranberry vines with an insect net. These larvae were brought to the laboratory and carefully separated from other insects and plant debris, placed on fresh cranberry plants, and kept under observation for three days. Any larvae that seemed sluggish in movement or abnormal in appearance were removed from time to time, so that chance inclusion of insects naturally infected with the disease might be reduced to a minimum.

In order to secure inoculation and infection, the following procedure was used: ten larvae were put in a straight-walled shell vial (eight centimetres by two and one-half centimetres in size), and while they clung to the bottom, the vial was inverted, and the mouth flamed and pushed down into a slice of potato, on which grew a pure culture of the fungus. Thus, a thin disc of potato was stamped out, which, when the vial was righted, served as a close-fitting stopper, on the lower surface of which the fungus continued to grow and produce conidia. The larvae, in crawling about the vial, either came directly in contact with the conidiophores and conidia, or the latter were shot off and fell upon them. In either case infection was satisfactorily accomplished, and, in one instance, a half-hour's exposure resulted in one hundred per cent. infection. When hundreds of larvae were so treated, however, only about half of them later became diseased; this failure to attain complete infection might in part have been due to natural immunity, but probably resulted from many larvae seeking the bottom of the vial and there quickly weaving a protective web, which shielded them from falling spores.

For the purposes of this investigation, three hundred larvae were inoculated between ten o'clock p.m. and one o'clock a.m., when conditions of temperature and moisture were most favourable for conidial germination. In order to provide a normal environment for the progress of infection, they were then removed to an artificial 'cranberry bog', made by filling a litre container with damp sand, into which cranberry uprights were thrust closely; melted paraffin was poured over the surface of the sand and the thin crust thus formed prevented evaporation from the sand, held the cranberry uprights firmly in place, and turned back any larvae that ventured down the stalks of the cranberry plants.

Use of the fungus in artificial culture by the methods outlined had made it possible so to control conditions that large numbers of larvae became inoculated at a known time. It was now desired to check the sequence of the disease at known intervals, and to study the course of infection step by step. In order to accomplish this, from four to eight inoculated larvae were removed from the cranberry plants at consecutive

two-hour intervals for three days and nights, anaesthetized with ether fumes, and killed and fixed in a formalin-acetic mixture (formalin, 10 c.c., glacial acetic acid, 5 c.c., and water, 85 c.c.), in which they were left for several days.

Further steps in technique involved washing the larvae in several changes of water, after which they were passed through a closely graded series of alcohols (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, and 90 per cent.), two hours in each grade; they were then left over night in 95 per cent. alcohol, followed by several hours in absolute alcohol with one change, and finally cleared in xylol (a graded series of mixtures of xylol and absolute alcohol, beginning with xylol 5 per cent., through 10, 20, 35, 50, 65, 80, 90, and finally 100 per cent.). Paraffin (50° C. melting point) was added to the xylol to the point of saturation, and the larvae were left in this paraffin-xylol mixture for several weeks, after which they were put in melted paraffin at 52° C. for twenty minutes, and embedded. Serial sagittal sections were cut, from 8  $\mu$  to 10  $\mu$  in thickness, and stained with Heidenhain's haematoxylin and eosin.

Study of these serial sections, from larvae killed and fixed at known successive intervals of time after inoculation, made it possible to follow step by step the progress of the disease and the concomitant sequence of changes in the tissues of the host.

#### DEVELOPMENT OF THE DISEASE IN RELATION TO THE INSECT.

In an earlier paper (6), the writer has described the morphology of *E. sphaerosperma* and has explained in detail how the conidia are discharged from the conidiophores. These conidia are projected into the air with some violence; they are elliptical in shape, and small enough (22  $\mu$  by 7  $\mu$ ) readily to lodge endwise among the cuticular denticles of the 'fireworm', where they are firmly held by the gelatinous apex of the spore. Indeed, while *E. sphaerosperma* attacks a variety of insects which have quite different cuticular surfaces, this particular host is peculiarly adapted to infection, because the shape, size, structure, and projectile-like flight of the conidia are all suited to lodge the spores most advantageously among its spines.

Under favourable conditions the conidia may germinate within one and one-half hours after discharge. The stout germ-tube rapidly dissolves its way through the external cuticle and the softer hypodermis below by the secretion of enzymes, as may be seen in Pl. XXVIII, Fig. 6, where the lighter-coloured clear area around the advancing germ-tube marks the region of enzymic activity.

Infection usually takes place through the dorsal surface, because this region is most exposed to conidia. The relatively very thick and hard black cuticle covering the head seems invulnerable; this may be because

its chitin is chemically different from other parts of the cuticle, or because spores cannot cling to this shiny smooth surface. All other portions of the body surface may be penetrated, and there seems to be no preference for the thinner areas between segments, as has been suggested in the case of some other insects. One or more nuclear divisions may occur while the germ tube grows through the body wall.

Some investigators have insisted that infection by entomogenous Entomophthoraceae occurs through the digestive tract. The writer has fed fireworms with the conidia of *E. sphaerosperma* (the larvae will feed avidly upon the fungus), and several hours later has killed, fixed, embedded, sectioned, and stained them; in no case had infection resulted, nor could germinating conidia be found in the alimentary canal. It seems, therefore, that infection in *Rhopobota* can occur only through the body-wall.

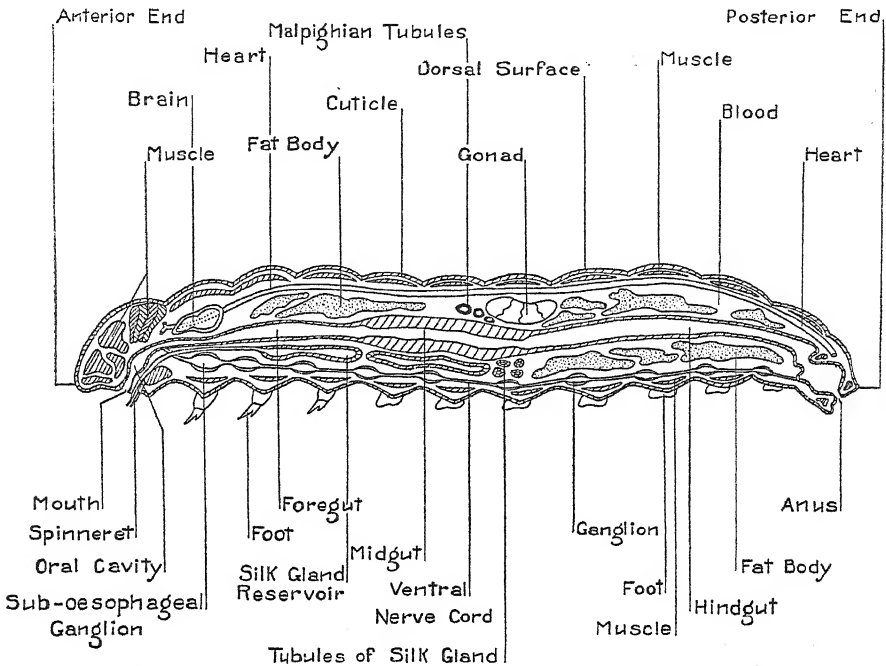
Before proceeding to details in the progress of infection, it will be well to review the general internal anatomy of *Rhopobota* larvae, as represented diagrammatically in the text-figure. First it should be noted that, in common with other insects, there is an open circulatory system, in which the only definite blood vessel is the dorsal tubular heart. Thus the colourless blood fills the body cavity, restrained only by the body-wall, and circulates freely among the organs whose surface it directly bathes.

The mouth leads back into a short, tubular foregut, behind which is the larger, elastic midgut (which may crowd all other structures into relative insignificance when distended with food), followed by a short and narrow hindgut. Associated with this digestive tube is the pair of salivary glands, which evolution has modified to function as silk glands, each with a narrow, convoluted secreting portion beside the posterior part of the digestive tube and, more anteriorly, a larger reservoir portion which in turn leads into a narrower duct that joins its fellow and opens to the exterior through the spinneret in the ventral lip.

Dorsal to the posterior part of the midgut lie the convoluted Malpighian tubules, excretory organs of the larvae. In the same region, but still more dorsally situated, is the relatively large oval gonad, with its developing reproductive cells. Striated muscles fill the head with oblique bundles that attach to the mandibles, while circular and oblique muscle bands form a lattice work beneath the hypodermis, surrounding the whole animal.

The large brain is dorsally situated in the head and has lateral connexions that join with the ventral nerve cord, which runs backward to the hindmost end of the body, with a swollen ganglion in each of the twelve segments. Packed in between the various other organs, wherever room may occur, are the lobes of the large fat-body, an organ of simple structure and complex function, the most obvious of which is the storage of fat.

Returning now to the course of infection in the larvae, the reader will note that because of the nature of the circulatory system, all conidial germ tubes that penetrate the body-wall must enter directly into the blood. In



Diagrammatic sagittal section through larva of *Rhopobota vacciniana*.  $\times 13$ .

this nutrient fluid growth is very rapid, and elongate, branched, multi-nucleate hyphae are quickly formed. Some of these hyphae segment into short, irregularly shaped pieces, which, floating freely in the circulating blood, are disseminated throughout the host and simultaneously start infection in different structures and regions of the body.

This general distribution does not result in the same rate of disintegration of the different structures attacked, however, and therefore a most interesting sequence occurs in the order in which different organs are destroyed. Development, after the initial growth in the blood, is most rapid in the fat-body and the associated oenocytes. The fungus penetrates the large thin-walled cells with great ease, destroying the tissue by enzymic action, as shown in Pl. XXVIII, Fig. 9, and Pl. XXVIII, Fig. 12, and quickly forming an extensively branching mycelium, whose hyphae penetrate this organ and the oenocytes in all directions.

Following the initiation of dissolution in the fat-body, other glandular tissues begin to show disintegration, including the hypodermis, the



silk-glands (salivary glands), and Malpighian tubules, as shown in Pl. XXVIII, Figs. 11, 13, 14, and 15. The destruction of foregut and hindgut follows, but it should be noted that the chitinous lining of these structures is left unimpaired. Indeed, chitinous structures within the larvae, including those mentioned, and the linings of the tracheae, do not seem ever to be attacked, although the fungus has demonstrated its ability to produce a chitin-dissolving enzyme when it penetrates the body cuticle. These different chitinous structures are, presumably, chemically similar, since they are all secretions of the same epithelium, and it is therefore difficult to account for this difference in solubility, unless the enzymes secreted by young germ tubes are somewhat different from those found in more mature hyphae. In any event, the digestive agents secreted by the fungous protoplasm are very potent, for with the exception just noted, all structures within the host are rapidly brought to a state of complete liquefaction.

The tissues next to be destroyed after the glands are the muscles. These structures, which in insects occur mainly in isolated strands, are structurally liable to rapid disintegration, because their form allows attack from all sides, and the fungus may not only penetrate, but also envelop, the structure (Pl. XXVIII, Figs. 10 and 11).

The nervous system is more resistant and remains unharmed when most other tissues, including the midgut, have begun to yield to the invading parasite; the brain and sub-oesophageal ganglion seem to be slightly less resistant than the ventral cord and its ganglia.

Of all tissues the developing gonad is most resistant, remaining unimpaired in the midst of almost complete dissolution of neighbouring structures (Pl. XXVIII, Fig. 17). It, too, finally succumbs to attack, and ultimately the only structures which retain their identity are the cuticular linings mentioned above, the external cuticle, and whatever remnants of undigested food may occupy the digestive tract.

These final stages in the destruction of the host are generalized and rapid. It would seem that when the fungus mycelium becomes well established in various organs, a superabundance of histolytic enzymes is produced, which become diffused through the already semi-liquid and partially disintegrated content of the body cavity, accomplishing a most thorough, complete, and rapid final dissolution. The host is now in a very soft, flaccid condition, in which the slightest touch may rupture the weakened cuticle and allow the content to flow out. It is during these last stages of disintegration that the fungus ceases vegetative development and turns to its reproductive phase.

It will have been noted that an interesting sequence occurs in the order of resistance to destruction shown by the various structures, which, in general, is in proportion to their relative activity, and therefore presumably to their relative rates of metabolism. Thus the fat-body, mainly a storage

organ and probably the most inert of the host's tissues, is most easily and quickly destroyed, while such active tissues as muscle and nerve persist the longest. Furthermore, the nuclei in the various kinds of cells resist disintegration longer than their cytoplasmic envelope, a condition which has often been observed in diseased tissues of both animals and plants, and has been attributed to the relatively greater metabolic activity of the nuclei.

Without regard to the individual organs concerned, development of the fungus and the consequent disintegration of the organ is usually most rapid in the thoracic region, and it is here that both rhizoids and conidiophores are likely to form first and appear externally upon the body of the host.

If, as is assumed above, it is true that the rapidity of development of the fungus is inversely proportional to the relative metabolism of the different organs attacked, then the fact just pointed out, namely, that the fungus usually develops most rapidly in the anterior segments of the larva, is directly opposed to the Axial Gradient Theory of Childs, according to which the fungus should develop most actively in the posterior region of the host's body, because it is there that the organs of lowest metabolism would be found.

The first external and macroscopic evidence that a *Rhopobota* larva is infected occurs only after the disease has been disseminated throughout its body and is well established in various organs. The larva now changes from its normal green colour to a somewhat yellowish hue, and makes restless, aimless movements which are quickly succeeded by the sluggishness that precedes death. The vitality of the host is remarkable, death occurring only after internal disorganization is well advanced, and the writer has found larvae alive and busily feeding when the disease had progressed so far that the midgut had been destroyed.

Within a few hours after death the organs of the host have largely become replaced by fungous mycelium, and the vegetative period of development is usually terminated by the segmentation of the mycelium into short, irregularly shaped hyphal bodies (Pl. XXIX, Fig. 19). This feature of the life cycle is not always the same, and seems to depend on whether the vegetative cycle is completed before the liquid nutrient materials of the host are exhausted; that is, hyphal bodies are produced most abundantly if the reproductive phase of the fungus is deferred until the hyphae begin to suffer diminution of nourishment. The latter seems to be the factor that stimulates hyphal segmentation.

After completion of vegetative development, the fungus may either fill the interior of the host with a mass of thick-walled resting spores, or may develop conidiophores, which burst through the thin cuticle in coalescing groups (Pl. XXIX, Figs. 21, 22, and 23), and later branch, thickly covering the host with a glistening white coat of conidia-bearing hyphae. As will be seen from Figs. 21 and 23 of Pl. XXIX, the developing conidiophores

exert considerable pressure, their ends becoming much swollen against the resistance of the enclosing cuticle.

The fungus develops rapidly in the small larvae of *Rhopobota* and the life cycle occupies but a brief time. The usual interval between inoculation with conidia and completion of the cycle by formation of conidiophores and conidia is about seventy-two hours.

#### SUMMARY.

1. *E. sphaerosperma* is a widely distributed and important member of the insect-destroying Entomophthoraceae.

2. The writer discovered the fungus upon a new host, *R. vacciniana*, and grew it upon artificial media.

3. Artificial culture offered new and never before equalled opportunities for controlled studies of infection and development of the fungous disease.

4. Inoculations with the fungus were made at known times, and the infected larvae killed and fixed at successive time intervals, whereby the sequence of the disease was followed, with special reference to its effect on the tissues of the host.

5. Infection takes place only through the body-wall, never through the digestive tract.

6. Conidia germinate, under favourable conditions, in one and one-half hours, and the germ tube penetrates the body-wall by enzymic dissolution in from two to twelve hours after germination.

7. Within the body, rapid growth takes place within the blood; the circulating blood is the medium by which metastasis of the disease is effected in the host.

8. Progress of the disease is marked by a definite sequence in the order in which different structures of the host are destroyed.

9. The fat-body and oenocytes suffer most rapid disintegration, followed by the silk glands, the hypodermis, and the Malpighian tubules, the foregut and hindgut, the midgut, the muscles, the nervous system, and the gonad.

10. Chitinous linings of the foregut and hindgut, and of the tracheae, are not attacked; and, after initial penetration by the germ tubes, no further dissolution of the chitinous cuticle is effected. The fungus never attacks ingested food materials.

11. The final stages in enzymic dissolution of the host by the fungus are most rapid and thorough, and with the completion of vegetative development, nothing remains of the original larva except the chitinous structures and remnants of food material mentioned above.

12. Diminution in available nutriment that accompanies the final stages of host destruction stimulates the segmentation of hyphae into hyphal bodies, and marks the end of vegetative development of the fungus. From these hyphal bodies may arise the masses of internal resting spores or the conidiophores which burst through the cuticle and complete the cycle by formation of conidia. Details of this morphology have been discussed by the writer in an earlier paper.

13. The host shows evidence of infection only after the disease has become well established throughout its body; the first symptoms are change from a green to a yellowish colour, and restless movements; these symptoms are followed by sluggishness and increased turgor. Death is deferred until near the end of vegetative development of the fungus, when the animal's tissues are much disintegrated and its body is soft and flaccid.

14. The average time necessary for the fungus to complete its cycle, from inoculation to conidial production, is about seventy-two hours.

This is the last of a series of three papers that have grown out of work done at Harvard University during 1928 and 1929. To Professor William H. Weston, Jr., head of the Department of Cryptogamic Botany, the writer hopes to convey some measure of his appreciation for that rare quality of enthusiasm that tinges every commonplace with inspiration, and that made this work a pleasure.

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## EXPLANATION OF PLATES XXVIII AND XXIX.

Illustrating Dr. W. H. Sawyer's paper on 'The Development of *Entomophthora sphaerosperma* upon *Rhopobota vacciniana*'.

In these plates the orientation of the figures, the organs and tissues shown, and the infecting fungus are uniformly denoted as follows:

A = Anterior end. P = Posterior end. D = Dorsal side. V = Ventral side. *a* = foregut. *b* = midgut. *c* = hindgut. *d* = brain. *e* = fat-body. *f* = silk gland. *g* = ventral nerve cord and ganglia. *h* = gonad. *i* = blood. *j* = cuticle. *k* = oenocytes. *l* = spinneret. *m* = sub-oesophageal ganglion. *n* = muscle. *o* = oral cavity. *p* = Malpighian tubule. *q* = nucleus of silk gland. *r* = heart. *s* = enzymic dissolution of tissues. *t* = nucleus. *u* = tracheary tube. *w* = food. *x* = fungous hyphae. *y* = conidium. *z* = germ tube.

## PLATE XXVIII.

Figs. 1, 2, 3, and 4. Sagittal sections of a *Rhopobota* larva, showing general anatomy of the host (cf. text-figure) and an early stage of infection by *Entomophthora sphaerosperma*, in which the fungus occurs only in the blood. × 63.

Fig. 1. Anterior end. The fungus is present as short, bud-like pieces in the blood, as seen at *x*, and as more elongate hyphae, as seen at *x'*.

Fig. 2. Region of midgut. The section is tangent to the posterior part of the stomach; thus the large digestive glands in the wall are shown at *b*. Portions of the fungus may be seen in the blood at *x*.

Fig. 3. Section in region behind midgut and through gonad. The reservoir portions of a silk gland show at *f*. At *c* may be seen the chitinous rings that line the hindgut. The gonad *h* is bordered posteriorly by parts of the fat-body *e*, and ventrally by cross-sections of the Malpighian tubules *p*. Portions of the fungus may be seen in the blood at *x*.

Fig. 4. Posterior end. The larva is curved laterally and the section is para-sagittal. Portions of the fat-body, cross-sections of the Malpighian tubules, and some muscle tissue are shown. Several fungous hyphae may be seen at *x*.

Fig. 5. A conidium *y* has lodged endwise among the spines of the dorsal cuticle and germinated; the germ-tube *z* may be seen penetrating the body wall. × 350.

Fig. 6. Detail of above, more enlarged. The conidium *y* contains three vacuoles, and a single vacuole is present in the tip of the germ tube *z*. The thin clear zone bordering the penetrating germ tube is due to dissolution of the body-wall by enzymic action. × 600.

Fig. 7. Detail from ventral side of Fig. 1 at point *j*, showing characteristic appearance of the fungus *x*, in the blood, during early stages of infection. × 350.

Fig. 8. Sagittal section in median dorsal region of larva. Nucleated coenocytic hyphae *x* may be seen lying lengthwise in the heart, of which the dorsal and ventral walls are indicated by *r* and *r'*. × 300.

Fig. 9. Penetration of the fat-body *e* by hyphae *x*. Oenocytes, large cells associated with the fat-body, may be seen at *k* and *k'*; the latter is penetrated by a hypha. × 300.

Fig. 10. Penetration of muscle *n* by hyphae *x*. Note the clear zone around the embedded hyphal threads, where the muscle tissue has been digested. At *j* may be seen the dorsal body-wall, and at *e*, on both sides of the muscle, portions of the fat-body; its large thin-walled cells are in intimate contact with the fungous hyphae, seen in cross-section and in side view. Thirty-two hours after inoculation. × 160.

Fig. 11. Malpighian tubule *p*, surrounded and partially destroyed by fungus. It will be noted that the surrounding tissue of the fat-body is filled with hyphae, and mostly disintegrated. The clear rounded bodies in the hyphal threads are vacuoles. Thirty-six hours after inoculation. × 180.

Fig. 12. Evidences of enzymic secretion. At *s*, ahead of the advancing hyphal tip *x*, the digestive substance secreted by the hypha has dissolved the fat-body, forming a cavity which is continued backward as a thin, clear zone all around the hypha. Fixation was very good in this specimen, and the appearance noted above is not due to shrinkage of the tissue. Twelve hours after inoculation. × 350.

Fig. 13. Malpighian tubule *p* invaded by hyphae and softened to the point of dissolution. The surrounding fat-body is almost totally destroyed. Thirty-two hours after inoculation.  $\times 350$ .

Fig. 14. Cross-sections of silk gland at *f* and muscle strands at *n*, during an early stage of attack by surrounding hyphae. Thirty-two hours after inoculation.  $\times 180$ .

Fig. 15. Silk gland *f*, partially disintegrated. Note the characteristic branched nucleus at *g*.

Fig. 16. Attack upon midgut. Note that neighbouring portions of the body are disintegrated, and hyphae have begun to invade the glandular wall of the stomach *b*. The ventral nerve cord and its ganglia, shown at *g*, have not yet been attacked, nor has the gonad *h*. Thirty-six hours after inoculation.  $\times 100$ .

Fig. 17. A well-advanced stage of infection. Note the abundance of hyphae which have developed in the now disintegrated fat-body, the partially destroyed Malpighian tubule *p*, and the intact muscle strand *n* and gonad *h*. The highly vacuolate condition of the hyphae is attendant on their rapid growth during this period of infection. Thirty-six hours after inoculation.  $\times 100$ .

#### PLATE XXIX.

Fig. 18. Brain, enveloped and penetrated by hyphae. Forty-four hours after inoculation.  $\times 320$ .

Fig. 19. Formation of 'hyphal bodies'. The vacuolate hyphae are in the midst of disintegrated fat-body cells and oenocytes. At *x* and *x'* may be seen the segmentation that resulted in the formation of the hyphal body between. A tracheal tube is shown at *u*. Thirty-six hours after inoculation.  $\times 350$ .

Fig. 20. An advanced stage of infection. The tissues of the host are now reduced to semi-liquid mass, much of which has been absorbed by the fungus, the hyphae of which are uniformly distributed through the insect; internal and external chitinous structures are all that remain intact of the host's tissues. Forty-four hours after inoculation.  $\times 70$ .

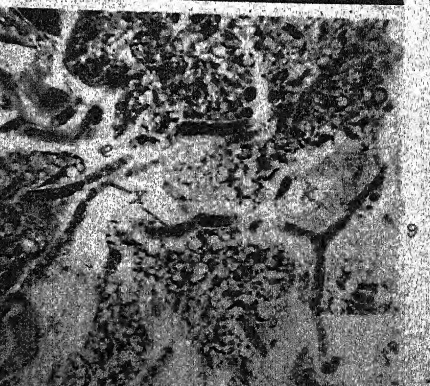
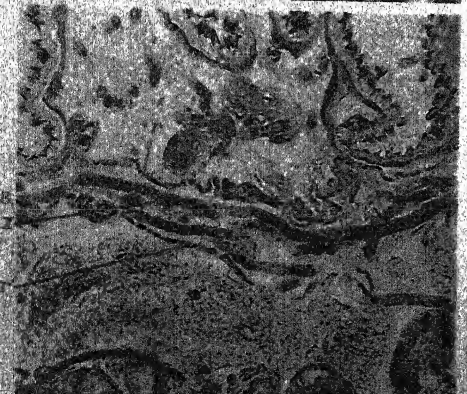
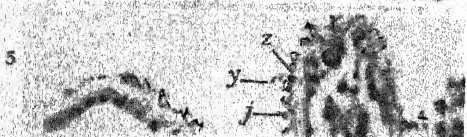
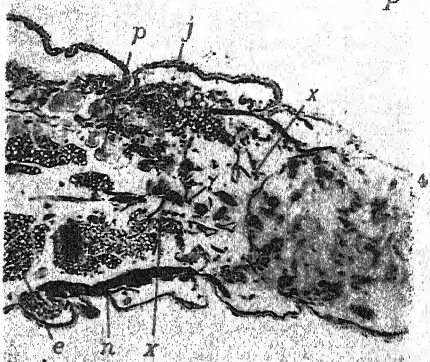
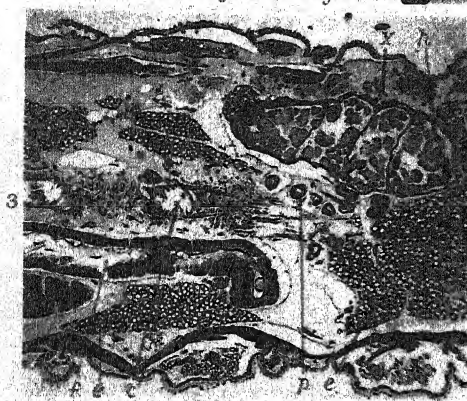
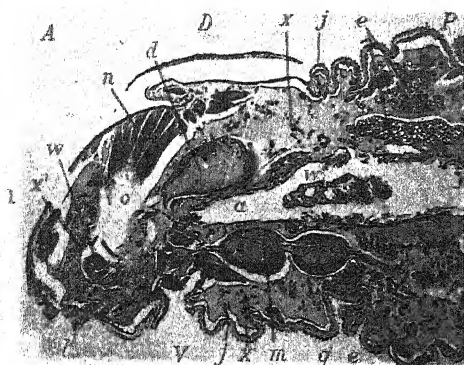
Fig. 21. Hyphae have aggregated in a group which is pushing against the dorsal cuticle, preliminary to bursting through to the exterior as functional conidiophores. Note how the resistance of the cuticle has caused the advancing hyphal ends to swell. The multinucleate character of the hyphae is clearly shown.

Fig. 22. Dorsal part of infected larva, showing its body filled with hyphae, and three groups of incipient conidiophores about to burst through the cuticle. Forty-four hours after inoculation.  $\times 126$ .

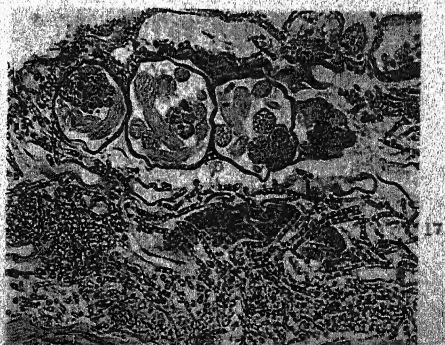
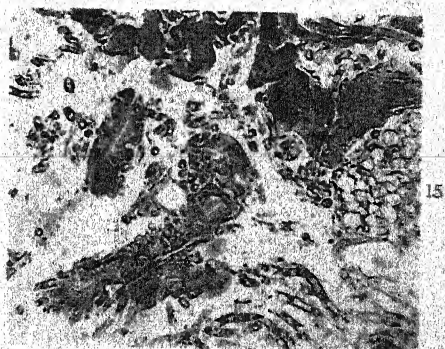
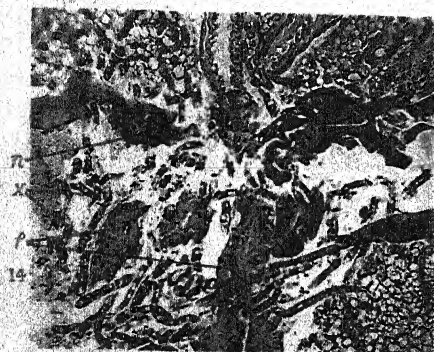
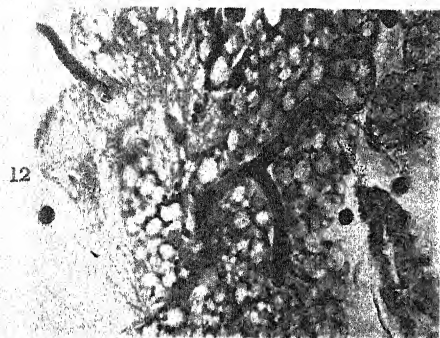
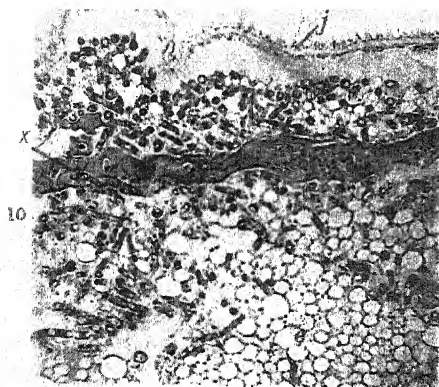
Fig. 23. A group of conidiophores similar to those shown in Fig. 4, in which nuclear division is taking place. The elongate nuclei seen at *x* are in a late stage of mitosis. Sixty-four hours after inoculation.  $\times 320$ .

Fig. 24. Another group of conidiophores. Note the narrow cone-shaped tip of the only one which has penetrated the resisting cuticle. Forty-four hours after inoculation.  $\times 320$ .

Fig. 25. Mature resting spores of *Entomophthora sphaerosperma*. The swollen, thin-walled bodies interspersed with the thick-walled azygospores are hyphal bodies, similar to those from which the resting spores were formed.  $\times 320$ .



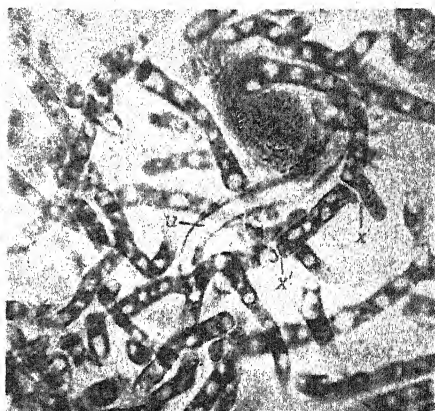




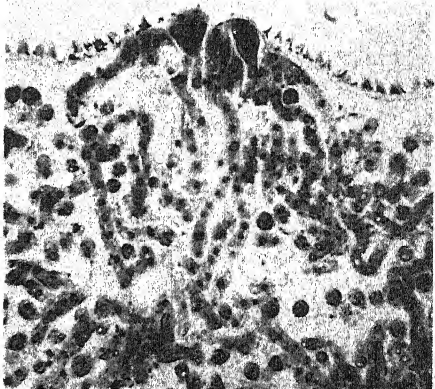
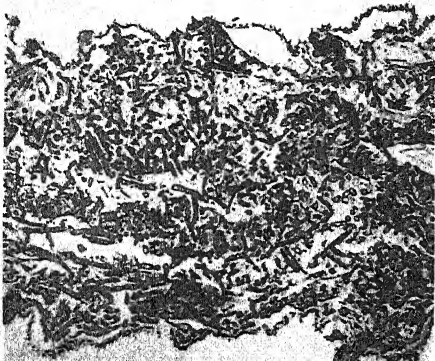




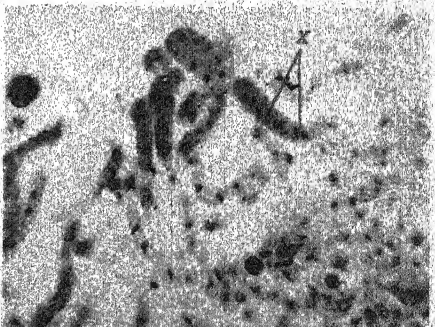
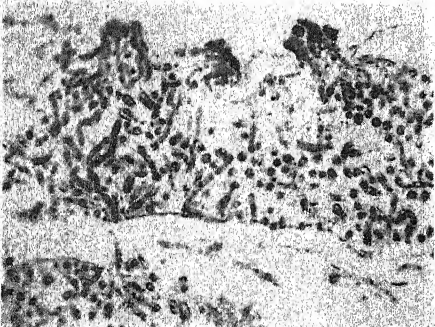
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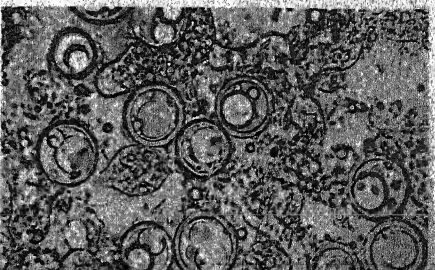
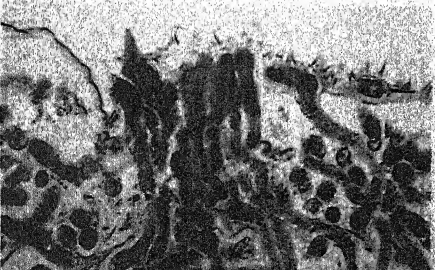
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# Chromosome Study and the Genetic Analysis of Species.<sup>1</sup>

BY

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THE cytologist approaches the study of species in two stages. In the first, he looks at the chromosomes of related plants and animals at mitosis. Comparison then shows him differences which he can explain by analogy with experimental data. If he finds, for example, one form with twice as many chromosomes as another, he infers that the first is tetraploid, and that it arose by a doubling of the chromosome number in some ancestor. He also infers that it may not cross with the diploid form and that if it does the progeny will be infertile. Further, if he is dealing with animal species he has reason to expect, on the analogy of various insects and crustaceans, that the tetraploid form (race or species) will prove to be parthenogenetic. If he finds, on the other hand, that one form has a pair of chromosomes more than the other and that this difference is accompanied by the appearance of new small chromosomes, as is the case in *Vicia* and *Fritillaria* and in many orthopterans, he assumes that fragmentation or the reciprocal process of fusion has occurred in an ancestor of one form or the other.

When a group of species have been examined in this way, it has usually been found that they fall into sections in accordance with the classification previously arrived at by the taxonomist. The main divisions of the taxonomist are sustained by the cytologist. It follows that, as there is this fundamental correspondence, the systematist can profitably make use of the discoveries of the cytologist, and thereby elucidate a number of problems which have defied analysis on taxonomic lines. Examples of such special problems are the cases of *Triticum* and *Rosa*, and *Drosophila*, where the distinction between the inter-sterile European *D. obscura* Fall., and the North American *D. pseudo-obscura* Frol., was first brought to light by a study of the chromosome complement.

But inferences from comparison of mitosis can never be as conclusive as the results gained in the second stage of investigation—the study of

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meiosis in hybrids between the related forms. Here the cytologist is able to apply rigorous principles to his interpretation (2). He assumes that association at metaphase occurs only between homologous parts of chromosomes—parts, that is, having the same arrangements of the same genes. He can infer, therefore, not only such changes as tetraploidy and fragmentation, but also others such as translocation, interchange (reciprocal translocation), reduplication, and inversion—changes in the relative position of segments of chromosomes which are not usually recognizable from the comparison of mitosis.

These changes in genetic 'structure' which distinguish related taxonomic forms have a special effect on meiosis. They interrupt the linear sequence of genes so that when the chromosomes pair at prophase they are not always, for spatial reasons, able to come together throughout the lengths where they are homologous, i.e. where their genes are identical (6). This leads to a reduction of crossing-over in the neighbourhood of such interruptions (5) and a reduction in the number of chiasmata which are formed as a result. The chiasmata are responsible for the pairing of the chromosomes at metaphase, and pairing therefore fails in a proportion of cases. Such is apparently the explanation of the partial or complete failure of pairing of chromosomes observed very generally in inter-specific hybrids. Thus it is found that even where pairing is perfect the number of chiasmata formed is reduced (1). And where it does fail, the frequency of pairing in different cells varies unimodally, as does the frequency of chiasma formation (2). Thus it is possible to conclude from the behaviour of hybrids that most related species differ in respect of the structure of their chromosomes.

In defining the differences between species it will be noticed that the cytologist and geneticist also define a hybrid as the product of the union of germ cells with dissimilar chromosomes. The taxonomist is accustomed to regard a form intermediate between two taxonomic types as a hybrid. While such a type is presumptive evidence of hybridization having occurred in the past, it is necessary to distinguish between several possible results of such hybridization. In the first place, the 'hybrid' may be a sterile first cross and of no importance in the life of the species, unless it is capable of reproducing asexually. In the second place it may be a pure-breeding derivative of such a cross, and have, like the experimentally produced tetraploid *Primula kewensis*, all the requisites of a new species. Such forms are *Spartini Townshendii* and *Aesculus carnea* (2). Further, we may recall that there are many forms that the taxonomist describes as species which the cytologist recognizes as hybrids. For example, many apomictic species of *Hieracium* are triploid and, therefore, if not hybrids between diploid and tetraploid species, at least the products of fusion of dissimilar germ cells, and in consequence sexually infertile. It may be

noticed parenthetically that cytology permits the study of these forms whose sterility necessarily defeats genetic analysis.

Again, there are diploid species which form rings of four or more chromosomes at meiosis. They breed true to this condition, which is evidently due to their being interchange-hybrids, owing to their homozygous progeny being non-viable. In *Hypericum* and *Rhoeo* the assumption rests on purely cytological evidence, but in *Oenothera* their hybridity can be shown by their producing twin-hybrids when crossed with other species. Since these species breed true, like the hybrid tetraploid species, they are permanent hybrids; they can survive and, it appears, have often survived the non-hybrid forms that gave rise to them.

In these ways the cytologist can specify certain changes and certain resulting conditions of hybridity that are associated with taxonomic distinctions. But how important are these changes? The taxonomist has long realized the importance of geographical isolation in permitting the independent development of new forms. Structural and numerical changes in the chromosomes determine another kind of isolation which is equally potent. They determine *genetic isolation*, for they always render the affected forms in greater or less degree intersterile. Thus, even if a tetraploid will cross with a diploid relative, their progeny will be triploid and infertile, while a new type with even a single interchange yields, in maize and *Pisum*, hybrids which are semi-sterile. Genes for cross-sterility (4) may also produce a similar effect, but their importance is unknown. We may conclude, therefore, that changes in the number and structure of chromosomes, while they do not as a rule establish those morphological differences which are the essential criteria of species, are nevertheless an important factor in permitting the development of such differences (by gene mutation) in the strains they isolate.

It follows from this conclusion that the same differences in structure and number of chromosomes that are found between species should also be found, although to a less extent, within species. This is, in fact, the case. Polyploid forms are known within forty species of flowering plants, and evidence of translocation, interchange, and fusion has been found in species of *Hesperotettix*, *Trimerotropis*, and other grasshoppers and moths, as well as in many plants.

These remarks make it clear that cytological and complementary genetical work can advance the study of species chiefly by the analysis of their internal constitution (3). This shows us that the simplest type of species is the diploid which is habitually self-fertilized. A more complicated type is the diploid which, owing to sexual differentiation or self-sterility, is habitually cross-fertilized. A third type is the sexually fertile polyploid species. A fourth, the mixed species which contains both diploid and polyploid forms. A fifth, the complex-heterozygote species

found characteristically in *Oenothera*. A sixth, the 'clonal species' which reproduces only by apomixis or vegetatively and whose character can often be determined from the fact that it is triploid and therefore incapable of normal sexual reproduction.

Genetics leaves no doubt that each of these types will have certain characteristic properties of variation. It is for the taxonomist, armed with the cytological information, to find out what these properties are.

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# The Effect of X-radiation on the Meiotic and Mitotic Divisions of Certain Plants.

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With four Figures in the Text.

## INTRODUCTION.

THE physiological reactions of living organisms and living cells to irradiation with X-rays and the emanations of radium have long been of interest to biologists. The subject has been studied from many aspects, but the growing use of these rays for the induction of heritable genetical and cytological changes calls for further study of the effect of irradiation on chromosome behaviour.

It has been amply proved both genetically and cytologically that the chromosomes may be altered under the influence of X-rays. These alterations may involve the mutation of one or more genes, the deletion or inactivation of portions of chromosome, or the fragmentation and subsequent translocation of chromosome segments.

Many of these changes are permanent and may be perpetuated in further cell-divisions, being finally transmitted to the progeny of the treated organism through the sex cells. On the other hand, destructive changes may be induced resulting in the death of the cell and the destruction of the chromosomes, but these changes are naturally of no genetical significance.

The present study is a description of the immediate and delayed effect of X-ray treatment upon the behaviour of the chromosomes at the mitotic and meiotic divisions of certain plants.

## PREVIOUS WORK.

The early literature upon the effects of X-rays, radium emanations, and other disturbing agents upon protoplasm and on the physiology of the living cell is extensive. For all aspects of the subject reference should be made to a comprehensive bibliography collected by Bersa (1) in 1927.



Only work connected with observations upon the effects of treatment on the chromosomes and their behaviour will be discussed here.

Strangeways and Oakley (9), and Strangeways and Hopwood (10), examined the immediate and delayed effect of X-ray treatment upon the mitotic divisions in cultures of the choroid and sclerotic tissue of chick embryos grown *in vitro*.

By carefully controlled experiments involving immediate and delayed fixation subsequent to various exposures to X-rays, these authors came to the following conclusions.

1. 'The earliest recognizable effect of X-radiation is the temporary inhibition of the onset of the mitotic division in the majority of those fully-formed cells which are about to divide.
2. Cells actually undergoing mitosis are unaffected by X-radiation.
3. It appears that vegetative cells pass through a phase immediately prior to visible prophase, during which the physiological processes of the cell are especially liable to be disturbed; if such a cell receives a dose greater than 30 e it will nevertheless enter mitosis, but the process of division will be of an abnormal type and may result in the complete disruption of the cell.'

More recently many authors have described experiments on these lines which confirm and extend the conclusions quoted above. It is quite impossible to review the extensive literature published in medical and radiological journals. Though the present author's observations on mitosis only confirm the results of many previous workers, they are included for direct comparison with the observations on meiosis.

The bulk of work on the effects of treatment on the reduction division has been carried out on animals and insects, and usually from the point of view of the cytoplasmic changes induced.

Goodspeed (3) describes the effects of treating pollen mother-cells of *Nicotiana Tabacum* with X-rays and radium when the treatment is applied during the late archesporial stage or during early prophase. He finds that this treatment gives rise to abnormalities involving fragmentation, non-disjunction and non-conjunction of the chromosomes, which changes first become apparent at anaphase of the first division. He concludes that the chromosomes are structurally but not visibly altered as the result of treatment, and that these alterations only become apparent when the chromosomes are subjected to the anaphase forces.

De Mol (7) describes a number of irregularities in the meiosis of tulips, induced by X-rays, but since no numerical data are given the observations are difficult to interpret.

Many authors have described chromosomal abnormalities induced by X-rays without considering their mode of origin.

Navashin (8) treated moistened seeds of *Crepis tectorum* with X-rays and obtained a range of dosage by varying the time of exposure. Examination of the root-tip divisions of the seedlings revealed a large number of abnormalities involving fragmentation and translocation. The degree of abnormality appeared to be proportional to the length of exposure.

Lewitsky and Araratian (5) obtained similar results by treating young seedlings of *Crepis*, *Vicia* and *Secale* spp. and fixing root-tips two days after treatment. The types of abnormality produced were similar to those described by Navashin. These authors record the fact that if root-tips are fixed immediately after treatment, the material is poor in mitoses and the chromosomes are shrunken and deformed. In more favourable plates the chromosomes are shortened and thickened but not structurally altered.

#### MATERIAL AND METHODS.

For observations on the effects of X-ray treatment upon meiotic behaviour the tulip (*Tulipa silvestris*) variety Philippe de Commines and the ornamental plant *Rhoeo discolor* were chosen. The chromosomes of both plants are large and easily fixed by the smear method. Philippe de Commines is one of the diploid varieties of tulip with twelve pairs of chromosomes and the behaviour at meiosis is normal. *Rhoeo discolor*, on the other hand, is abnormal, in that segmental interchange has led to the formation at meiosis of a ring, or one or more chains of chromosomes united end to end (Darlington (2)).

For the effects of treatment upon somatic mitosis *Crocus Olivieri* was chosen, as the chromosome complement consists of only three easily distinguishable pairs of chromosomes.

All drawings were made with the aid of a 1.5 mm. oil-immersion objective, a compensating eye-piece ( $\times 30$ ) and a camera lucida, at an initial magnification of about 6,000 diameters.

##### *Tulipa.*

A large batch of Philippe de Commines was grown in the open during the spring of 1932. The plants were lifted on June 8 when the first samples of ten bulbs each were treated. Subsequently further samples were taken from store at intervals of about a month and treated in the same way.

A Coolidge type water-cooled X-ray tube was used, and the intensity of irradiation was kept constant for every treatment, only the length of exposure being varied. The tube was operated as follows:

90 kilovolts, 5 milliamps, 30 cm. target distance. Unscreened irradiation. The treatments are summarized in the following table, and for convenience each treatment will in future be referred to by its number.

Exposure.	June 8.	July 11.	Aug. 17.	Sept. 19.	Oct. 25.
3 min.	1 a	2 a	3 a	4 a	5 a
5 "	1 b	2 b	3 b	4 b	5 b
7 "	1 c	2 c	3 c	4 c	5 c

The majority of the garden tulips undergo the reduction division during the latter half of September. Examination of untreated bulbs on September 20 showed the pollen mother-cells to be in all stages of division.

Smear preparations were made of P.M.C. from untreated bulbs and from bulbs taken from the treated samples as they became ready. The preparations were fixed in medium Flemming and 2BD (4). The latter fixative gave the best results at all stages. The material was stained by the gentian-violet-iodine method.

Meiosis was delayed by treatments 1, 2 and 3, and owing to the fact that the original samples were rather small, sufficient material could not be spared for the successful fixation of material from every treatment. The following dates upon which the material was suitably fixed will give some indication of the delay of meiosis caused by the different treatments:

Sept. 20	.	.	.	control, 4 a, 4 b, 4 c
Sept. 26	.	.	.	1 a, 1 b, 1 c
Oct. 7	.	.	.	2 b, 3 a
Oct. 10	.	.	.	3 b.

3 c was several days later, but sufficient material could not be spared to obtain divisions.

The material of treatment 5 was not fixed, as the anthers contained young pollen when the treatment was applied.

#### *Rhoeo discolor.*

A plant of *Rhoeo discolor* with buds at all stages of development was selected and treated as follows:

64 KV, 5 ma., for 15 minutes.

The average distance of the inflorescences from the target was approximately 30 cm. Irradiation was unscreened.

Immediately after treatment, smear preparations were made of pollen mother-cells as described for *Tulipa*. After an interval of 24 hours further preparations were made. Preparations made previously from untreated material were used as control.

#### *Crocus Olivieri.*

Corms of this species were planted in fibre, and when well rooted two pans were treated as follows:

- A. 64 KV., 5 ma., 15 min., 30 cm. target distance,  
 B. " " 30 " " " " "

The small amount of fibre covering the roots would not seriously affect the penetration of the rays.

Immediately after treatment a well-rooted corm from each pan was carefully lifted, washed, and placed in a Petri dish containing water. Root-tips were fixed from both at intervals. The fixative used was 2 BE, which proved very satisfactory.

Root-tips were fixed as control immediately before treatment. Details of the times of fixation of treated material are given below.

No.	Treatment.	Period between treatment and fixation.
1	A	30 min.
1 a	B	30 "
2 a	A	7 hours
2 b	B	7 "
3 a	A	24 "
3 b	B	24 "
4 a	A	48 "
4 b	B	48 "
5 a	A	72 "
5 b	B	72 "

Root-tips from each fixation were sectioned in paraffin wax at a thickness of 20  $\mu$ , and stained by the gentian-violet-iodine method.

#### OBSERVATIONS.

##### *Tulipa.*

*Treatments 1a, 1b, and 1c.* A large number of cells were examinedd at all stages from diplotene to telophase of the second division, and no sign of any abnormality was observed.

*Treatment 2b.* Again many cells were examined, and in one only a fragment was observed lagging at second telophase. The remaining cells appeared quite normal.

*Treatment 3a.* The majority of divisions appeared normal, but occasionally a single fragment was seen at metaphase, and in one cell at late anaphase.

*Treatment 4a, 4b, 4c.* Observations were made at random in this material. Though the degree of the abnormality was to a certain extent proportional to the dosage, the type of abnormality encountered was the same in all.

Fixation appeared to be affected by these treatments, and though the structure of the bivalents was fairly clear at diplotene and very clear at diakinesis in many cells, metaphase plates were crowded and the bivalents somewhat shapeless. At diplotene the existence of fragments in some cells was suspected, but owing to the thinness of the chromosomes at this stage no certainty could be attached to the observations. At diakinesis, however, fragments were clearly observed in several cells, and in one it was possible to interpret twelve bivalents and a fragment. In one of the

bivalents a free arm was missing and corresponded in size with the fragment (Fig. 1). At metaphase approximately the same proportion of cells had small fragments as at diakinesis.

The earliest stages of anaphase cannot be made out, but it is clear from later stages that the anaphase separations of the chromosomes are



FIG. 1. Twelve bivalents and a fragment at diakinesis in *Tulipa*. N.B. The last bivalent lacks a free end, corresponding in size with the fragment.  $\times 2,000$ .

abnormal. A proportion of chromosomes will separate normally while the remainder lag. At a slightly later stage, a number of fragments of different sizes are seen and the separating chromosomes are drawn out into threads (Fig. 2). The bulk of the chromatin reaches the poles, but some remains on the plate and appears to disintegrate. The metaphase of the second division is much disorganized, and when the chromosomes separate at the second division complete disorganization sets in, and though the bulk of the chromatin reaches the four poles a large number of fragments are left on the plate, most of which disintegrate.

#### *Rhoeo discolor.*

Examination of material fixed immediately after treatment showed no trace of abnormality. Fixation was good, and all stages from diakinesis to the formation of tetrads were observed and differed in no way from the control.

Material fixed twenty-four hours after treatment yielded results very similar to those observed in *Tulipa*. The chromosomes at metaphase were shrunken, and in many cases the threads forming the terminal chiasmata were drawn out. Beyond this the cells were normal, with one exception, in which a bead of chromatin was seen to lie between the ends of two adjacent chromosomes at metaphase (Fig. 3a). No such phenomenon has been seen before in *Rhoeo*, and it may well be that the formation of this bead indicates a weakening of the chromosome and that the bead itself may become one of the fragments seen at anaphase.

Fragments and disintegrating chromatin were observed at anaphase, and the appearance of the divisions from anaphase to the formation of tetrads exhibited precisely the same type of disorganization as that observed in *Tulipa* (Fig. 3b).

#### *Crocus Olivieri.*

The somatic chromosomes of *C. Olivieri* have been illustrated by Mather (6). A normal metaphase has however been drawn in Fig. 4a for

comparison with abnormal divisions. The three chromosome types are readily recognizable from the position of the attachment constrictions. Great care was taken during the observations to make certain that the



FIG. 2. *a*, anaphase in *Tulipa* showing fragmentation and lagging bivalents. *b*, late anaphase. *c*, late second division anaphase showing lagging fragments and disintegrating chromatin.  $\times 2,000$ .



FIG. 3. *a*, part of the ring of chromosomes at metaphase in *Rhoeo discolor* showing an abnormal bead of chromatin attached to the ends of two chromosomes. *b*, telophase of the first division showing lagging fragments.  $\times 2,000$ .

chromosomes had suffered no damage from the knife in cutting. Fixation was good, and the structure of each chromosome could be made out in the majority of the divisions.

The observations from each fixation derived from two or more root-tips, in each case are summarized in the following table:

No.	Period between treatment and fixation.	Observations.
1	—	Mitoses plentiful and entirely normal
1 a	30 min.	Mitoses plentiful, all normal and indistinguishable from control
1 b	30 min.	Mitoses plentiful, in some divisions the chromosomes were shortened and thickened, but otherwise normal
2 a	7 hours	No mitoses
2 b	7 "	" "
3 a	24 "	" "
3 b	24 "	" "
4 a	48 "	Mitoses scarce, a proportion being abnormal
4 b	48 "	No mitoses
5 a	72 "	Mitoses plentiful, a large proportion being abnormal
5 b	72 "	Mitoses plentiful, the majority being abnormal and the degree of the abnormality being greater than in 5 a

It will be seen from the above that while the chromosomes actually undergoing division are not visibly affected by X-ray treatment, cell-division is rapidly retarded and is brought to a standstill for a definite



FIG. 4. *a*, normal somatic metaphase of *Crocus Olivieri*. *b*, *c*, *d*, abnormal plates induced by treatment.  $\times 2,000$ .

period of time, the extent of which is determined by the length of the exposure. Growth then starts again slowly, and from the beginning abnormal mitoses are observed. As growth becomes more vigorous the degree of the abnormality increases. However, the above observations agree with those of other authors, and it is unnecessary here to describe in detail the observed abnormalities induced in *C. Olivieri* as they were of the same type as those described in great detail by Lewitsky and Araratian (5), involving fragmentation of one or more chromosomes and more rarely translocation. Three abnormal plates, together with a normal division, are illustrated in Fig. 4.

#### DISCUSSION.

The observations on the effects of treatment on the root-tip divisions of *Crocus Olivieri* agree closely with those of Strangeways and his colleagues, whose conclusions were quoted earlier. A physiological reaction is set up, which, although it allows mitosis already in progress to finish and form daughter cells, inhibits a resting cell from entering mitosis. After a certain period growth starts again and abnormal divisions are seen. These authors imply that such divisions are the first undergone by nuclei treated while at rest, but do not make it clear whether the mitoses which have finished normally will proceed normally in future divisions. The fact that all activity was suspended for a considerable time in the *Crocus* root-tips, and that a large proportion of the cells seen in division after recovery were abnormal, indicates that in addition to the reaction, leading to the suppression of division in resting nuclei, those cells in division when treated, though completing the division normally, may well give rise to abnormalities in the next generation.

It appears likely that the sequence of events is as follows. X-ray

treatment causes a relatively spontaneous physiological reaction, which is sufficient to suppress further division in resting nuclei but not able to stop the process of division already begun. This is in agreement with Strangeways and Hopwood (10), but it is possible to go further and suggest that this change increases slowly in intensity, and when the intensity is at its highest acts in a similar way upon the resting nuclei, derived from divisions allowed to proceed normally after treatment. Activity is suspended for a certain period (depending upon the dose applied), and then the effect wears off and mitoses begin to appear. Many are abnormal, and it is clearly possible that these abnormalities may have been initiated during the post-mitotic as well as during the pre-mitotic resting stages following treatment.

The following observations upon the meiotic behaviour after treatment tend to confirm this extension of Strangeways and Hopwood's hypothesis. The main points brought out by these observations are as follows:

(1) There are no immediate visible effects of treatment, with the exception of clumping and contraction of the chromosomes (probably due to delay between treatment and fixation).

(2) In the material fixed twenty-four hours after treatment the stages up to metaphase are relatively normal, though a number of cells (in *Tulipa*) contained fragments at diakinesis and metaphase. From the beginning of anaphase to the telophase of the second division the behaviour is extremely abnormal.

(3) The type of abnormality observed involves the collapse and some disintegration of the chromosomes, and in no way resembles the more simple changes observed at mitosis.

(4) Treatment one month before metaphase of meiosis, when the cells would have been in very early prophase, though checking the process considerably yields normal final stages of meiosis with the exception of occasional fragments seen at all stages.

(5) Treatment applied two and three months before the metaphase of meiosis, when the tissue would probably be undergoing somatic mitosis, causes no meiotic abnormality (one exception only was seen, in which a fragment was observed lagging at second anaphase).

We have to seek an explanation of this behaviour and to correlate the observations at meiosis with those at mitosis.

The essential difference between the two divisions is undoubtedly due to the difference in the time taken for the completion of the cycle of division. Mitosis is a rapid process taking only a few hours, while the extended phases of the meiotic division may take days, or even weeks, to reach completion. At mitosis the chromosomes are allowed to complete their division before the induced physiological change has reached its highest intensity. The cells undergoing meiosis on the other hand are caught by



the peak of the induced change at every stage of division. The division in progress is not brought to a standstill (otherwise no second division stages would be seen twenty-four hours after treatment; they would either be held in interphase or in tetrads), and the induced change acts upon the chromosomes at all stages and not only upon resting nuclei.

The chromosomes probably become temporarily altered physiologically, and it is only the stresses to which they are subjected at anaphase while in this condition, which renders this previous alteration visible in the shape of fragmented and disintegrating chromosomes. The division is not stopped (though its speed may be affected) and passes abnormally through interphase and second division to give rise to the extremely abnormal conditions observed at second anaphase. Chromosomes at late metaphase or anaphase when treated would probably complete the division normally, but cells at earlier stages when treated would give rise twenty-four hours later to the observed abnormalities.

Cells which do not reach anaphase in twenty-four hours after treatment appear relatively unaltered. The existence of occasional fragments at diakinesis and metaphase in *Tulipa* is difficult to explain, but is probably due to a delayed action induced by the rays on the thin chromosomes. If the action were direct, fragments would have been observed at these stages in fixations immediately after treatment. None were seen, but it is possible that fragments due to direct action of the rays on early diplotene would be missed in observation.

The delay in reaching the final stages of meiosis in bulbs treated one month before fixation indicates a considerable induced check to the progress of the division. The cells at the time of treatment would have been in very early prophase, and the fact that the later stages of division are relatively normal shows that chromosomes treated at this stage are able to recover before being subjected to strains at anaphase, and yield normal divisions. The few fragments seen may have been initiated by the direct action of the rays, but it is more likely that they arose as the nuclear contents were released from the induced check and normal activity was renewed.

The fact that meiosis in bulbs treated two or three months previously is (with the exception of one cell) entirely normal, shows that even if abnormalities are induced in the somatic tissue giving rise to the P.M.C. they are short-lived and are probably eliminated at the expense of normal tissue. This is supported by the fact that, although the morphology and physiology of the tulip plant is altered by treatment, any induced change is not permanent. The offsets from treated bulbs when grown in the following season give rise to normal plants. The one abnormal cell may have escaped elimination as the result of its comparative normality.

When these observations and conclusions are compared with those of

Goodspeed (3), it will be seen that while the final results of treatment and their interpretation are very similar, there is an important difference. Goodspeed obtained abnormal final stages of meiosis by treatment of archesporial tissue, and of pollen mother-cells in early prophase. Treatment at this stage in *Tulipa*, however, yielded normal final stages, with rare exceptions.

If the whole meiotic cycle in *Nicotiana Tabacum* is considerably shorter than that of *Tulipa*, then the hypothesis put forward in this paper may well apply to Goodspeed's results.

It is fully realized that such terms as 'physiological reaction' and 'physiological change' are vague and unsatisfactory. Many hypotheses have been put forward to account for the observed changes induced in living cells by irradiation. None are entirely satisfactory, and it is beyond the scope of this paper to attempt to discuss them.

#### SUMMARY.

The effects of previous X-radiation upon meiosis in the tulip (*Tulipa silvestris*) Philippe de Commynes and upon *Rhoeo discolor*, and on the mitosis in *Crocus Olivieri* are described.

It is observed that:

- (1) Treatment has no immediate visible effect upon either division.
- (2) Treatment applied at a considerable period before meiosis (1-3 months) does not seriously affect the meiotic behaviour, though occasional fragments are seen at late stages.
- (3) Treatment twenty-four hours previously induces destructive changes at meiosis, which first become apparent at anaphase.
- (4) Mitosis, following treatment, is brought rapidly to a standstill for a period, after which abnormal divisions occur involving simple fragmentation and translocation.

It is suggested that a physiological reaction is induced which grows in intensity and then diminishes. This reaction prevents cells about to divide from entering mitosis but allows those already in mitosis to complete division, owing to the rapidity of the process. The cells are therefore submitted to the most intense period of the reaction during an enforced resting stage. The abnormalities are initiated at this stage.

The effect of this reaction upon chromosomes at meiosis alters them, so that if they are subjected to the stresses at anaphase during their temporarily altered condition, abnormality involving destruction will result.

The more simple changes involving fragmentation are induced in those cells which do not reach anaphase in their altered condition.

The essential difference between the induced abnormality at meiosis and mitosis is therefore due to the difference in time taken by the division to reach completion.

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# Callus Formation in *Hibiscus Rosa-sinensis* L. and *Hevea brasiliensis* Müll. Arg.

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With Plates XXX-XXXIV.

## CONTENTS

	PAGE
I. INTRODUCTION . . . . .	827
II. ROLE OF THE CAMBIUM IN REGENERATION OF TISSUES . . . . .	828
III. PRESENT INVESTIGATION . . . . .	829
IV. METHODS . . . . .	830
V. DEVELOPMENT OF CALLUS FROM EXPOSED WOOD SURFACE AFTER STRIPPING . . . . .	830
(a) <i>Hibiscus Rosa-sinensis</i> .	
(b) <i>Hevea brasiliensis</i> .	
VI. CALLUS FORMATION IN THE BARK OF <i>H. Rosa-sinensis</i> . . . . .	832
VII. CLEFT-GRAFTS OF <i>H. Rosa-sinensis</i> . . . . .	833
VIII. DISCUSSION . . . . .	835
IX. SUMMARY . . . . .	837
LITERATURE CITED . . . . .	838

## I. INTRODUCTION.

THE subject of callus formation is one which impresses the investigator who undertakes a study of the literature with a notable lack of definition. The probable reason for this is that the main approach to a study of the subject has been along pathological lines; the complex tissue growths following on insect attacks, as in galls, force themselves into prominence, and have been the subject of innumerable investigations. Again, callus formation is one of the most significant phenomena in the regeneration of tissues after wounding, and as the mechanism of regeneration differs

considerably according to the tissue system affected, the subject is likely to be a complicated one. In this article an account is given of a simple mechanism of callus formation which is perfectly clear cut; it is of interest to note that it is the same, apart from minor details, in two plants which are not closely related.

## II. THE ROLE OF THE CAMBIUM IN REGENERATION OF TISSUES.

It will not serve any useful purpose to make a comprehensive review of the literature, but it will be advantageous to draw attention to abstracts which indicate the present position and the importance usually attached to the role the cambium is understood to play in tissue regeneration by formation of callus. For the purpose of this paper the following points require consideration:

- (a) Regeneration of tissues about wounds.
- (b) Regeneration of tissues in grafting.
- (c) Regeneration of the bark on stripped surfaces.

The following abstract (2) gives a résumé of the position in relation to (a) and (b):

‘Among the functions of the cambium is the formation of callus or wound tissue and the healing of wounds. When wounds occur in roots and stems, masses of soft parenchymatous tissue quickly form on or below the injured surface; this tissue is known as callus; callus may be formed by the division of parenchymatous cells in the phloem and cortex, but its most frequent source is the cambium. In the formation of callus in the healing of a wound there is first abundant proliferation of the cambium cells with the production of masses of parenchyma.’

‘The important practices of budding and grafting have, as their basis, the ability of the cambium of both stock and scion to develop callus and unite, thus forming over the union of stock and scion a continuous cambium layer which will give rise to normal conducting tissue. The whole matter of cambium activity and structure in relation to the graft union needs further careful study.’

‘When the cambium is injured during the growing season as, for example, when branches are ringed, the cambium may be regenerated from the immature xylem cells beneath, provided the tissues are protected from desiccation soon after the injury. Thus, in ringing experiments it is sometimes difficult to prevent the formation of new cambium even by scraping the surface of the wound with a knife. In such cases, callus tissue is formed by the living immature cells of the xylem, and in this callus a new cambium is differentiated.’

With reference to (c) the following abstract clearly states the position (1):

'The *cambium* or layer of tissue exposed when bark is stripped is extremely delicate and composed of cells which by dividing give rise to new wood inwardly and new bark outwardly. The renewal of bark on a stripped surface depends wholly upon the activity of the cells of the thin layer of cambium. The mere rubbing of the exposed layer is sufficient to destroy the cells of which it is composed; the action of a disinfectant solution will destroy it; and rain water pouring over the surface or exposure to direct sunlight will destroy it. There seems, however, to be a considerable variation among trees in their power to build up new bark on stripped surfaces.'

As far as the writers can judge, the above abstracts fairly reflect the position as generally understood,\* and prominence is usually given to the important role which the cambium plays in the regeneration of tissues. It is our intention to show clearly that, in at least two cases, the cambium plays singularly little part in the reconstitution of damaged tissues until a comparatively late stage, all the primary phases of development taking place quite apart from the cambium.

### III. PRESENT INVESTIGATION.

The present investigation commenced as a study of the development and union of the callus tissues during the operation of bud-grafting on *Hevea brasiliensis* Müll. Arg. A short report has already appeared (5).

When following the various stages in the process of union of stock and scion in *H. brasiliensis*, some difficulty was experienced because of the formation of tannin-like deposits in the callus cells, which stained intensely with most dyes and resisted the usual destaining methods. The presence of these tannin cells tended to obscure details, so a search was made to obtain a more favourable plant for study. *Hibiscus Rosa-sinensis* L. is a common hedge plant in Malaya, and it proved particularly favourable. This plant forms alternate layers of hard and soft bast in the bark, and the latter can easily be peeled from the stem in layers of varying thickness. This proved of considerable importance, for the behaviour of the medullary rays throughout the inner bark could be clearly observed. The clear-cut, alternate rows of hard and soft bast gave strikingly clear pictures of the process of regeneration by wound callus. In addition, grafts could easily be obtained with this plant, either in the laboratory or by the usual horticultural practices, whereas under the same conditions grafts of *Hevea* invariably failed. As the work on *Hibiscus* progressed, the probability of a similar process of regeneration taking place when the bark tissues of *Hevea* are stripped from the wood became obvious, and as the practice of 'stripping' had been strongly recommended in the past for treatment of trees suffering from Brown Bast and is still practised for control of Patch

Canker, an exact study of the mechanism of replacement of stripped areas on *Hevea* stems was undertaken.

#### IV. METHODS.

The *Hibiscus* stems selected for stripping were growing vigorously and about one inch in thickness. For the study of the behaviour of the medullary rays in the wood two vertical parallel cuts half-an-inch apart were made in the bark, penetrating to the wood. A third incision joining the two vertical cuts at the upper end was made, and the edge of the bark gently lifted from the wood, pulled downwards and broken off. The rectangular area of wood so exposed was protected from desiccation by a bandage of waxed tape wound round the stem with an overlap above and below of one inch.

For the study of the behaviour of the medullary ray cells in the phloem, sections of stems six inches long were cut, incised as described above, but the panel of bark was merely reflexed and held away from contact with the wood by a suitable wedge. These stem pieces were then placed and kept in a moist atmosphere with the lower end dipping in about half-an-inch of water. Cleft-grafts were also made with six-inch pieces of stem and placed in a damp atmosphere as above. Controls were kept by making grafts on trees growing under natural conditions.

The earliest and succeeding stages in callus formation were obtained by fixing material every day for a week and afterwards at four-day intervals. Bouin's solution was used throughout for the ordinary histological work. Material for the study of nuclear division was fixed in weaker Flemming's solution or acetic alcohol. The material was embedded in celloidin and sectioned at 15–20  $\mu$  thick. General staining was obtained by a combination of carbol fuchsin and cotton blue, and cambial development was best demonstrated by Delafield's haematoxylin alone. Nuclear phenomena were studied by Heidenhain's staining method with iron-alum-haematoxylin. Other combinations tried were gentian violet and orange G, carbol-thionin and Bismarck brown, and satisfactory preparations were obtained with all of them. For the study of nuclear behaviour and early phases of cambial development, the developing callus tissue was carefully stripped from the wood and embedded in paraffin.

#### V. DEVELOPMENT OF CALLUS FROM THE EXPOSED WOOD SURFACE AFTER STRIPPING.

(a) *Hibiscus Rosa-sinensis* L.

(b) *Hevea brasiliensis* Müll. Arg.

(a) *Hibiscus Rosa-sinensis*. When the wood of a vigorously growing stem of *Hibiscus* is exposed by stripping the bark covering, the bark tongue still remaining attached at the base, the separation takes place at the

cambial zone, a region several cells thick radially and consisting essentially of meristematic cells derived from the cambial initials. The exposed surfaces of both wood and bark, therefore, are meristematic tissues.

The first signs of activity on the wood surface exposed by the removal of the bark patch become manifest two to three days after stripping. In transverse sections it will be seen that the end cells of the medullary rays, which in *Hibiscus* are generally one to three cells wide, have grown out into large, rounded, or oval-oblong cells with thin walls and sparse cytoplasmic contents (Pl. XXX, Fig. 1). The nucleus is embedded in a central mass of cytoplasm from which delicate threads pass to the periphery of the cell. Between neighbouring rays, smaller cells of similar structure develop from the meristematic cells normally destined to form xylem elements. As a result of these activities, the surface of the wood becomes quickly covered with a layer of thin-walled cells, the most striking feature of which is their enormous size when contrasted with those from which they are derived. This proliferation of primary callus from the wood is accompanied by a similar development from the cut edges of the bark bounding the wound (Pl. XXXII, Fig. 8). Here the cell elements are more varied in character, but with the exception of the hard bast elements all may take part in callus formation. As, however, the elements of the ray system preponderate, it is reasonable to assume that, as in the wood proliferation, they give rise to the bulk of the bark callus. The development of this callus does not proceed far before an extension of the phellogen downwards inhibits further growth. Thus, at the present stage, the entire wounded surface is covered with callus tissue, the base of the cavity being covered with callus derived from the medullary ray-cells of the wood, the edges being covered with callus of bark origin.

The proliferating phase above described is generally completed in about six days from the date of stripping, and a further increase in thickness of callus tissue at the base of the cavity is brought about by normal nuclear and cell divisions. Repeated mitosis, followed by tangential wall formation, results in a more or less radically disposed series of cell-rows, loosely aggregated at first (Pl. XXX, Fig. 2), but later, by mutual pressure, becoming consolidated into a large-celled, parenchymatous cushion about 1 mm. thick covering the face of the wood (Pl. XXX, Fig. 4 B). The cushion does not fill the cavity, the depth of which naturally varies according to the thickness of the bark, but which in the present instance was 4 mm. The phellogen already developed within the bark callus mentioned above, now rapidly extends from the periphery, inwards, just beneath the exposed surface of the callus cushion, the thin layer of cells thus cut off to the outside becoming suberized to form a protective layer to the delicate tissue beneath. In this way the phellogen is completely restored (Pl. XXXI, Fig. 6).



This second phase of callus formation occupies fifteen to twenty days. During this period the normal activities of the uninjured stem have proceeded as usual. Secondary thickening has resulted in an appreciable increase in diameter of the wood, excepting the area devoted to callus formation, in consequence of which the callus appears to be sunk in the wood to a depth equivalent to the thickness of the newly added xylem. The cambial ring has also been carried outwards a similar distance, so that the severed ends now impinge on the radial faces of the callus some little distance from the original position at the time the bark was stripped.

The third phase which now ensues concerns the development of a new cambium across the callus. This, as in the phellogen, commences at points where the severed ends of the old cambium impinge on the edges of the callus, and like a slowly closing diaphragm sweeps gradually inwards until the opposing edges meet and the cambial cylinder is thus restored (Pl. XXXI, Fig. 6). But even before the cambial cylinder is fully closed, the earliest formed cambium appearing in the callus tissue has begun to function normally, so that at the twenty-fifth day the characteristic layers of hard and soft bast begin to make their appearance at the periphery (Pl. XXXI, Fig. 7.) The subsequent history is merely one of normal cambial activity. In younger stems, where the bark does not exceed 2 mm. in thickness, complete regeneration is accomplished in a period of two months.

(b) *Hevea brasiliensis*. The facts, as established in the previous section for *H. Rosa-sinensis* are essentially the same for *H. brasiliensis*, except that in the latter the earliest stages of proliferation and division are more deep-seated. In the former case, the terminal cells of the medullary rays proliferate directly above the general level before any division takes place, but in *Hevea* other cells below the terminal cells commence to proliferate and divide before there is any extrusion beyond the level of the wood surface (Pl. XXX, Fig. 5). As a result, a group of dividing medullary ray cells grows out above the general level, and by the continued divisions and union of these cell groups, ultimately the callus cushion is built up. The contribution of bark callus and the development of a protective layer is exactly similar, and again there is not the slightest sign of cambial activity until a continuous and compact layer of callus, derived almost entirely from medullary ray cells, is laid down.

## VI. CALLUS FORMATION IN THE BARK.

It has been pointed out that when a slip of bark is reflexed but not detached from the parent stem, callus formation takes place on the inner face. Here, again, the terminal cells of the phloem medullary rays rapidly proliferate (Pl. XXX, Fig. 3) accompanied, as in the wood callus, by proliferation of the cambial meristem detached at stripping. The picture is, in

fact, a mirror image of the primary wood callus and, in like manner, increase in thickness is brought about. It has not been possible to determine the maximum amount of callus that can be developed, for invariably the bark strip has died in the course of a few days. It sometimes happens that the bark fails to strip at the cambial zone, and layers of tissues of varying thickness are left in contact with the wood. In transverse sections of such material (Pl. XXXII, Fig. 8) the protean character of the bark elements is well shown; the vigorous ray proliferation is again strikingly evidenced, but where areas of the soft bast are exposed, smaller cells comparable to those previously described are evolved from the phloem parenchyma. Where the fan-shaped expansions of the rays merge into the narrow layer of parenchyma external to the phloem the whole tissue is potentially functional.

#### VII. CLEFT GRAFTS OF *H. ROSA-SINENSIS*.

In order to define clearly the areas responsible for callus formation, and to evaluate the respective contributions of scion and stock to the union, it was decided to culture the two units separately. The stocks were split radially for about an inch and the scions trimmed to the usual narrow wedge shape with a sharp knife and placed in a moist chamber for four days.

By the third day, the developing callus appeared as a pale green velvety coat, completely covering the cut surfaces of the bark of the split stock. In addition, a fine line of callus could be made out following the line of junction between wood and pith. The cut end of the stem presented two concentric zones of callus tissue, a broad zone covering the bark and a narrow zone enclosing the pith. Except that in the obliquely trimmed scion the pith is exposed only in the thinnest part of the wedge, callus production is exactly as described in the stock.

In transverse sections, these areas are more clearly delimited. The medullary rays on entering the phloem widen out to form funnel-like wedges between the phloem masses, and extend outwards almost to the phellogen. The component cells are of large size and tangentially elongated. The phloem consists of stratified layers of hard and soft bast interrupted radially by the passage of the narrow ray elements; the cortex exterior to the phloem is but two to three cells deep. It is probable that the phloem and other parenchymatous cells both take an active part in callus production, but as the latter form a comparatively small proportion of the bark it is evident that the main source of callus resides in the medullary ray system which constitutes possibly two-thirds of the available plastic material. Pl. XXX, Fig. 4 A gives a very clear picture of the proliferation from the expanded ends of the medullary rays in the phloem.

Pl. XXXIII, Fig. 12, is a transverse section of a four days' cultured stock, and shows clearly the vigorous proliferation and division from the radially cut faces of the medullary rays and the narrow zone of cambial meristem. This is repeated in the narrow zone of small celled tissue bounding the pith, into which the protoxylem elements project. This latter would seem to be of minor importance, as although in young stems the pith is of comparatively large bulk, it disappears rapidly with the increasing age of the stem. It is apparent therefore, that practically the whole body of callus, produced to a large extent by proliferation and division of medullary ray cells, is of phloem origin.

Pl. XXXII, Fig. 9, is a transverse section of the scion wedge, which displays the same general features as those observed in the stock. As, however, the pith is exposed only in the thinnest part of the wedge it is evident that the callus contribution from this bounding zone is negligible.

With the callus-producing areas clearly defined as above, further progress in the union of the stock and scion must be the result of a progressive infiltration of callus tissue into the space between them. The contribution of stock and scion towards the formation of the callus is of approximately equal volume. This is well shown in sections of twelve-day grafts (Pl. XXXIII, Figs. 10 and 11). In Fig. 11 the contribution shown by the scion from the obliquely shaved bark areas is very striking. The complete infiltration of the lacunae may be attained in a month under favourable conditions, but two months may be required. Vigour, age of stock and scion, and meteorological conditions are the controlling factors. An indication that union has been attained is the shooting of the resting buds of the scion, and this has been observed as early as three weeks from the time of grafting. The histological features at this stage are illustrated in Pl. XXXIII, Fig. 13. Infiltration is complete and a solid cushion of callus fills each of the two fissures bounding the scion, extending from epidermis to pith. The individual cells of the callus tissue are intimately fused with each other and with the varied cell elements of stock and scion along the faces of the fissures. This makes the anatomical union complete throughout the graft.

The establishment of a cambial bridge across the callused gaps between stock and scion quickly follows the union; its evolution closely follows that described for bark regeneration, i.e. the prior development of a protective layer by an extension of the phellogen, followed later by the ingrowth of the cambium across the callus wedges. By the end of the second month the cambial cylinder is completely restored and secondary thickening is in progress. A phellogen is developed in alignment with that of the stock and scion, and the callus cells become strongly lignified in the areas bounded by the wood. By the end of the fourth month the continued activity of newly formed cambium has resulted in the deposition of a considerable

mass of wood and phloem (Pl. XXXIV, Fig. 14). The appearance of these characteristic masses of stratified phloem gradually restores the gap in the bark to uniformity.

#### DISCUSSION.

The controversial subject of the origin of callus tissue in grafting operations, either from cambial elements or from medullary ray elements, is a very ancient one, but, as far as we are able to gather from the literature at our disposal the subject still remains open for discussion. Kostoff (3) published an article in 1928 in which he calls attention to the fact that Goppert, in 1874, described the joining tissue between scion and stock as a product of the medullary rays. He also points out that Sorauer questioned this statement. Kostoff makes no contribution on this point and contents himself by stating that the joining tissue between stock and scion is usually a product of the stock. That this statement cannot be entirely accepted we have shown in the case of cleft-grafts of *H. Rosa-sinensis*, where the scion contribution to the callus cushions is quite as prominent as the stock component thereof.

The fact that proliferating medullary ray elements play a part in callus formation in many types of plants has been long known. With reference to *H. brasiliensis*, Rutgers (6) recorded in 1918 the proliferation of medullary ray cells in branch injuries effected by lightning, and more recently, Mann and Gunnery (5) have indicated the importance of the proliferating medullary ray cells in the operation of bud-grafting. But it is doubtful if the true significance of the formation of the callus bed from medullary ray cells in the so-called 'callus formation by the cambium' has been fully appreciated up-to-date. When discussing this subject Küster (4) described in the building of the new tissue that the cambial cells divide in the same way as under normal conditions, and that sections through very young callus tissues show that in respect of the direction of the early cell divisions there is no deviation from the normal.

The photographs submitted clearly show that the above explanation is quite unsatisfactory in the two cases investigated, for there is a very big deviation from the normal, the wood and bast-forming cambium of the stem taking little or no part in the earliest stages of callus formation, as described herein. The formation of a covering layer on stripped surfaces, and the packing of the interstices between stock and scion when a graft is made, is the result of the proliferation of parenchymatous elements, a very great preponderance being derived from the medullary rays. There is no suggestion of cambial activity of any description in the wounded areas, until the bed of callus tissue is fully laid down. The mechanism is so simple and clear cut in both cases that it seems possible that this type of tissue regeneration will be found to be the common method in woody stems

for the following reason. The medullary ray system functions normally both in a storage and translocatory capacity, and is situated in such a position that any wounding influence will probably directly affect the system. Thus when large increases in nutrient materials are required for accelerated development, as is the case when rapid regeneration of tissues is taking place, all the immediate necessary factors are concentrated in the medullary ray system. This claim cannot be made for any other tissue system in the plant, and therefore the suggestion made above receives some support from such a consideration.

The two functions of medullary ray callus tissue which are considered to be of primary importance are :

(a) as a joining tissue by which a passage is formed for the supply of nutrient materials from the soil through the stock to the scion, and

(b) as a source of supply of nutrient materials for the rapid growth of regenerating cambial elements.

Function (a) is the role of immediate urgency and the rapid production, close packing, and interlocking of the callus cells with the tissues of stock and scion result in the rapid building of a passage so that nutrient materials from the soil pass to the scion, from which source of supply it is cut off temporarily. While this role may be of immediate urgency it is by no means of premier importance. The storage and translocatory function as outlined in (b), together with the ideal situation of the medullary rays, provides the plentiful source of supply which is demanded by any rapidly developing tissue system such as arises when the cambial ring begins to sweep inwards to effect the completion of the wood and bast forming cambium. This nutritional function, we consider, is of far greater importance than any other.

The cushion of callus tissue obviously functions as a mechanical support upon which the cambial ring is later fully reinstated. This function, however, cannot well be separated from the nutritional one. The new cambial elements do not appear in the callus until the cushion is fully formed, and the complete laying down of the foundation tissue before the appearance of ingrowing cambial elements both from the phellogen and the cambium suggests that new cambial elements can only develop from previously formed cambial elements, and therefore that the cells of the callus tissue do not form component parts of the reconstituted cambial ring. Alternatively, it may be suggested that the cambial elements in contact with the callus cells on each side of the injury stimulate the adjoining callus cells into active division so as to finally form a complete cambial ring. We cannot give definite support to either suggestion, but the statement can be made that new cambial elements do not appear except in close proximity to pre-existing cambial elements. The gradual closing of

the insweeping cambial ring, starting from the points of severance at the sides, is very striking, more especially as these points have moved for some distance outwards owing to continued development of the uninjured portion of the cambial ring, and consequent growth in thickness. This feature is very noticeable in cleft-grafts where the scion is of less diameter than the stock; the insweeping cambial ring starting from the stock then dips down for a considerable distance through the callus bed in order to effect union with the cambium developing from the scion.

The consideration of the structure of the cells of the callus tissue formed over stripped surfaces, or during grafting operations, affords a simple explanation of many features which have previously been attributed to the failure of the development of a sensitive meristematic cambial layer. The callus is composed of thin-walled turgid cells which would lose water easily by desiccation. This explains the absolute necessity for a constant high atmospheric humidity if regeneration is to be carried out successfully. Any injurious agency will materially retard callus development, for thin-walled tissues of this type are usually very sensitive to changes in environmental conditions, and any external interference, more especially as regards insufficient supplies of moisture, must profoundly affect the proliferation and development of the initial covering layers.

This work opens up a fertile field of enquiry, but our investigations cannot be carried further owing to the exigencies of the present situation. The study of the origin and union of the cambia of the same species, as in the cleft-grafts of *H. Rosa-sinensis*, naturally leads to the investigation of the origin and union of cambia in grafts where stock and scion are of different clones, varieties or even species, and many new facts would undoubtedly be obtained. Further, the study of the exact mechanism of recovery of the injured tissues of woody Dicotyledonous plants would be worth while in order to gain a true picture of the actual facts.

#### SUMMARY.

1. The so-called callus formation by the cambium has been studied in *Hibiscus Rosa-sinensis* and *Hevea brasiliensis*.
2. Studies of callus development on stripped surfaces on both plants, and cleft-grafts of *H. Rosa-sinensis* show that the cambium takes no part in the early development of the callus cushion which is formed predominantly from the medullary ray system.
3. The formation of the callus cushion and subsequent development is seen in its simplest form on stripped surfaces. The predominant part played by the medullary ray elements in the production of the callus cushions is emphasized.
4. There is no sign of cambial activity until the callus cushion is completely laid down.

5. Both bark callus and wood callus are developed largely from medullary ray elements, and their respective contributions towards the formation of the callus cushion are pointed out.

6. It is shown in cleft-grafts of *H. Rosa-sinensis* that the contribution of callus tissue by the scion is approximately equal to that made by the stock.

7. The origin of callus tissue from the bark with a smaller contribution from the zone bounding the pith is shown in cleft-grafts of *H. Rosa-sinensis*.

8. The commencement of cambial activity at the points where the ends of the severed cambial ring impinge on the callus cushion, and the ingrowth of the ends of the cambial ring till the two unite in the middle of the callus cushion are described.

9. The functions and situation of the medullary rays lend support to the view that the simple method of tissue regeneration described herein, will be found to be a very common method in woody plants.

#### POSTSCRIPT.

Since this paper went to press observations germane to the present investigation have been published by J. E. Sass (Formation of Callus Knots on Apple Grafts as Related to the Histology of Graft Union, *Bot. Gazette*, xciv, 364, 1932).

Sass' observations concerning the tissues responsible for callus formation and the differentiation of a cambial bridge across the callus and also his views on the minor importance of the cambium in wound reactions are in general agreement with those expressed in the present paper. Sass, however, did not observe any proliferation from the medullary rays in the xylem or from the perimedullary zone; consequently he ascribes callus formation exclusively to tissues outside the xylem cylinder.

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# EXPLANATION OF PLATES XXX-XXXIV.

Illustrating Mr. A. Sharples' and Mr. H. Gunnery's paper on 'Callus Formation in *Hibiscus Rosa-sinensis* and *Hevea brasiliensis* Müll. Arg.'

## PLATE XXX.

Fig. 1. Proliferation of end cells of the medullary rays in the early formation of wood-callus (*H. Rosa-sinensis*).  $\times 300$ .

Fig. 2. Later phase of callus formation; *c.c.* showing radially disposed callus tissue developing from bark tissues; *w.c.* callus developing on surface of wood (*H. Rosa-sinensis*).  $\times 90$ .

Fig. 3. Proliferation of medullary ray cells on inner face of bark when the latter is stripped (*H. Rosa-sinensis*).  $\times 225$ .

Fig. 4. A. *p.c.* Proliferation of cells from the ends of the expanded medullary ray in the bark (*H. Rosa-sinensis*).  $\times 100$ . B. Large celled parenchymatous cushion of callus cells consolidated by mutual pressure. *c.c.* callus developed from bark tissues; *w.c.* callus developed over surface of the wood (*H. Rosa-sinensis*).  $\times 100$ .

Fig. 5. Proliferation and division of deep-seated medullary ray cells in the earliest stages (*H. brasiliensis*). Note dark-staining deposits in the actively growing and dividing cells.  $\times 330$

## PLATE XXXI.

Fig. 6. Completed phellogen but incomplete cambium reconstruction; *i.c.* unjoined ends of incompletely restored cambial cylinder; *c.p.* complete phellogen layer (*H. Rosa-sinensis*).  $\times 28$ .

Fig. 7. Formation of characteristic layers of hard and soft bast about the twenty-fifth day (B.L. = Bast layers) (*H. Rosa-sinensis*).  $\times 28$ .

## PLATE XXXII.

Fig. 8. *p.c.* Proliferation of primary callus from medullary rays passing through bark tissue overlying the wood. *c.c.* Proliferation of callus cells from radial faces of the bark tissue (*H. Rosa-sinensis*).  $\times 50$ .

Fig. 9. Transverse section of scion wedge in the early stages. *p.c.* callus developed from zone bounding pith; *c.c.* callus developed from bark.  $\times 32$ .

## PLATE XXXIII.

Fig. 10. Infiltration of callus tissue from both stock and scion; *c.c.s.* bark callus from stock; *c.c.sc.* bark callus from scion; *c.p.z.* callus from zone bounding pith.  $\times 55$ .

Fig. 11. Longitudinal section of Fig. 10. Note relative amounts of callus contribution.  $\times 18$ .

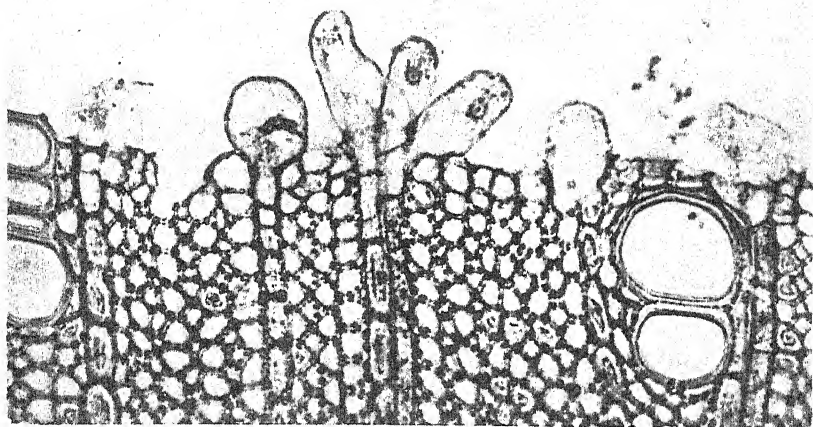
Fig. 12. Proliferation of medullary ray cells in the bark, and of cells from the zone bounding the pith after four days culture.  $\times 100$ .

Fig. 13. Final union of stock and scion with interstices filled by callus cushions. No cambial development across the callus cushion at this stage.  $\times 75$ .

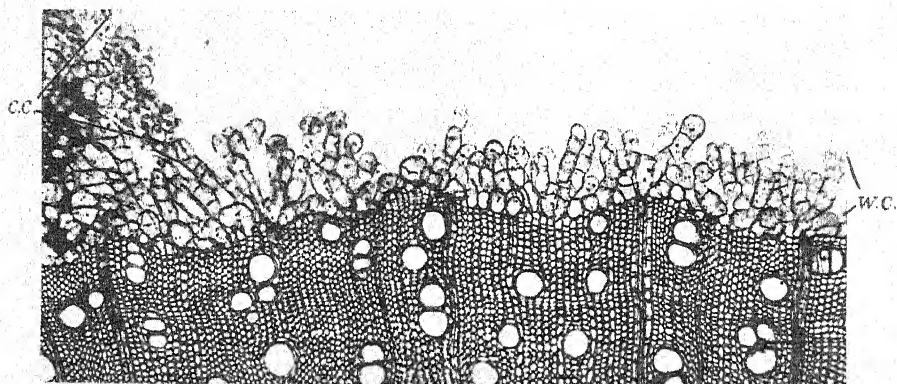
## PLATE XXXIV.

Fig. 14. Complete union of stock and scion after six months. Secondary tissues already well developed from the cambium in the callus cushion.  $\times 21$ .

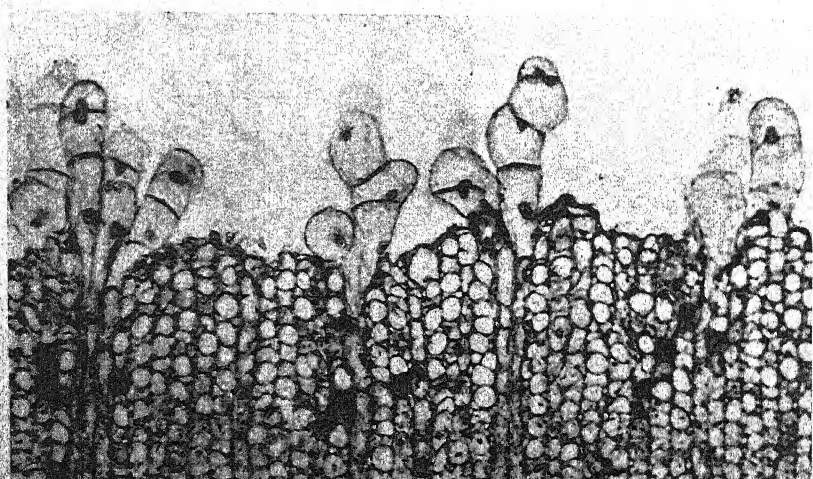


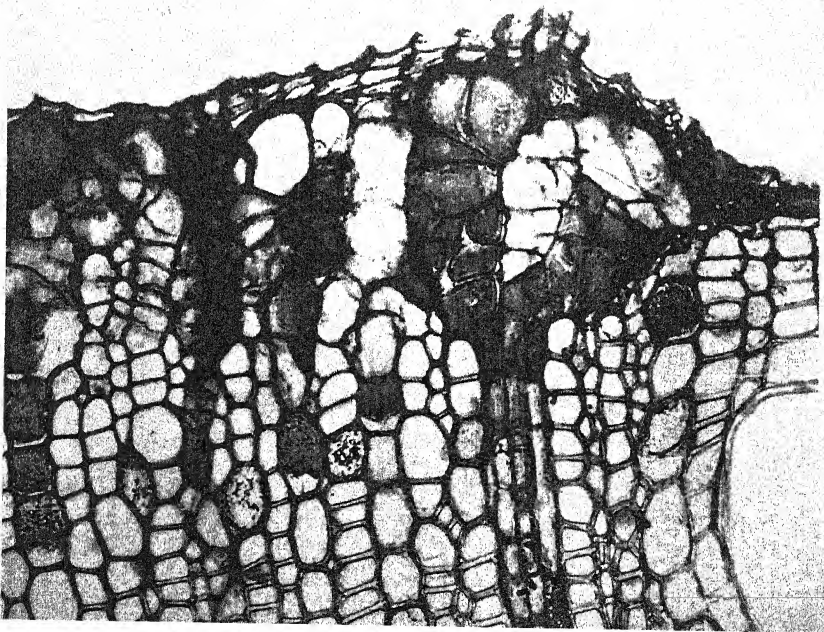


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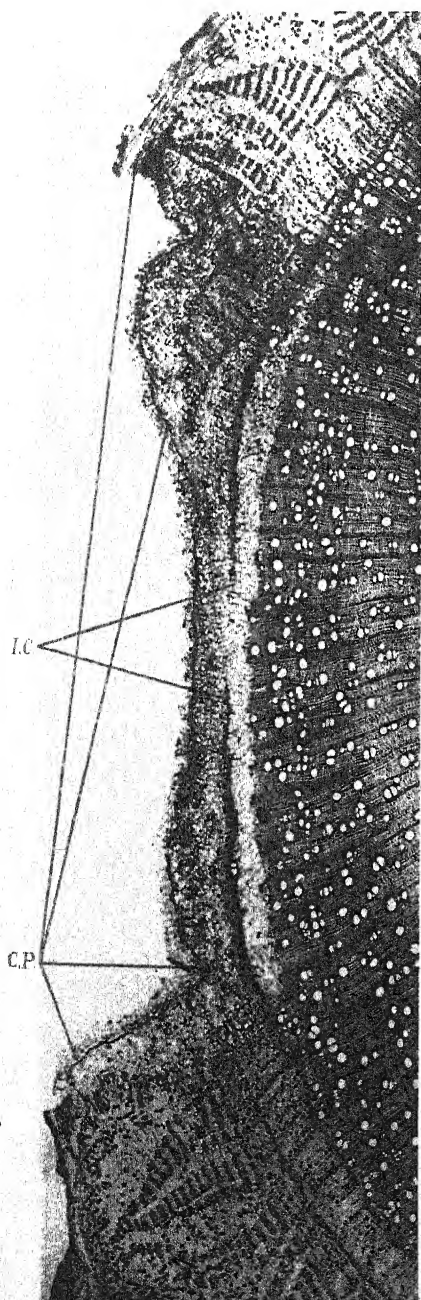


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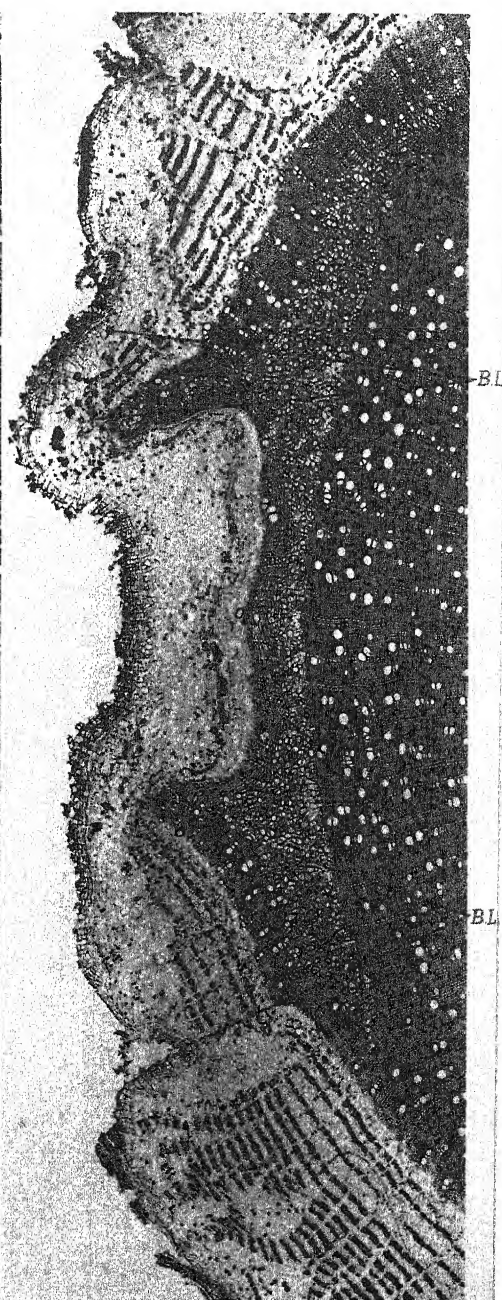






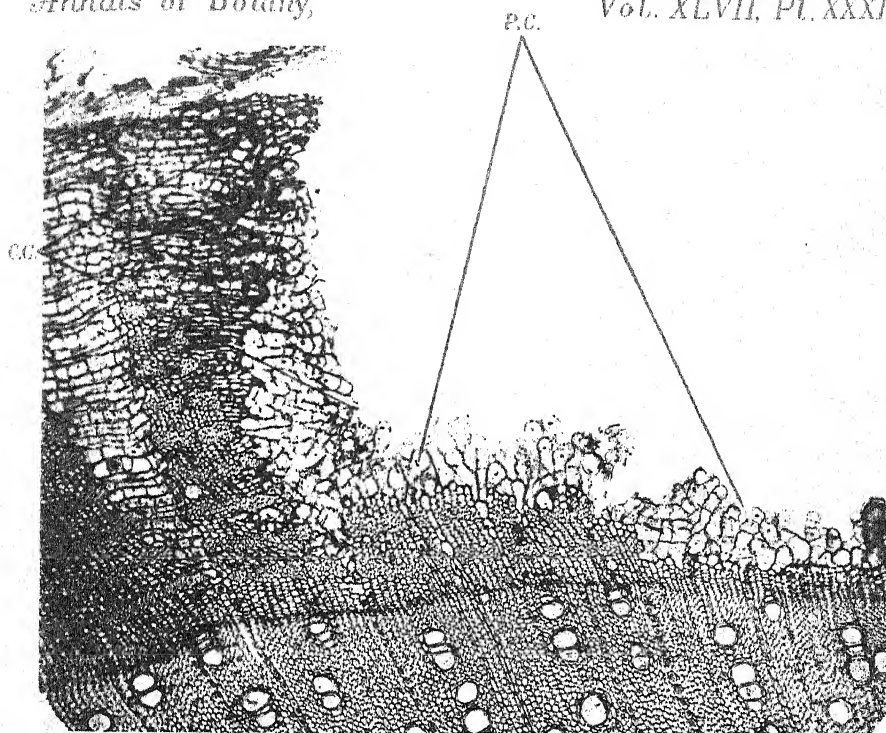


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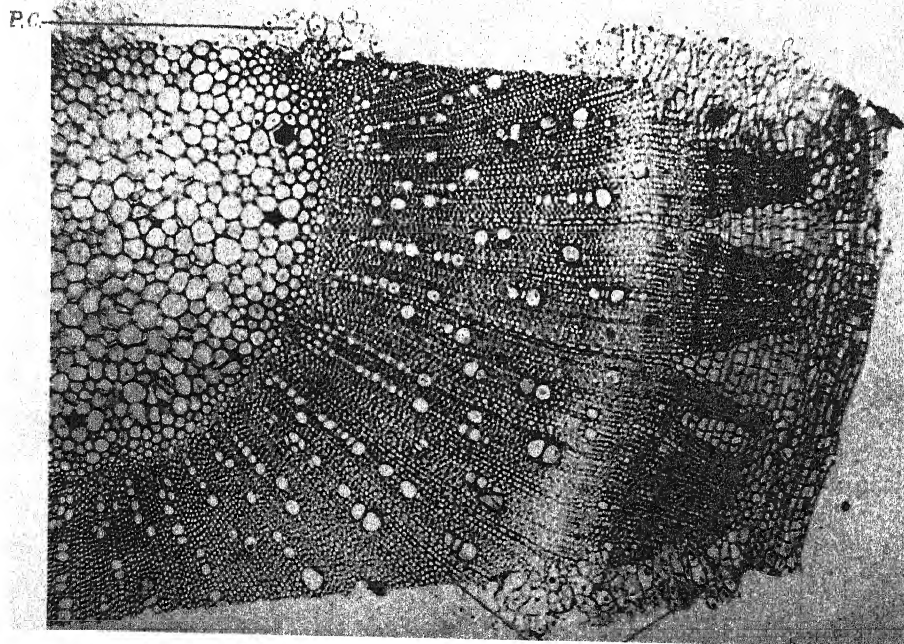


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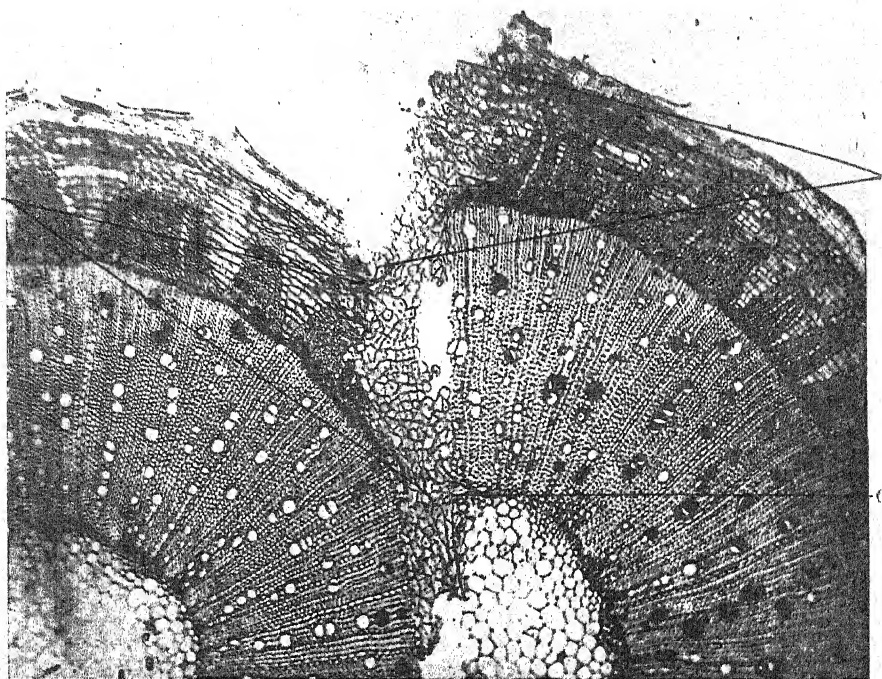


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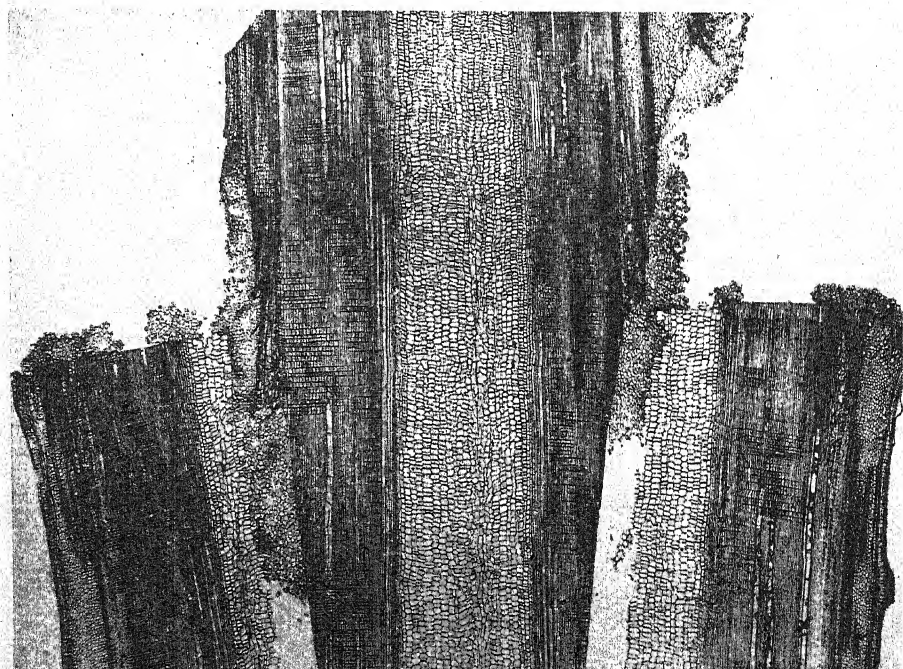


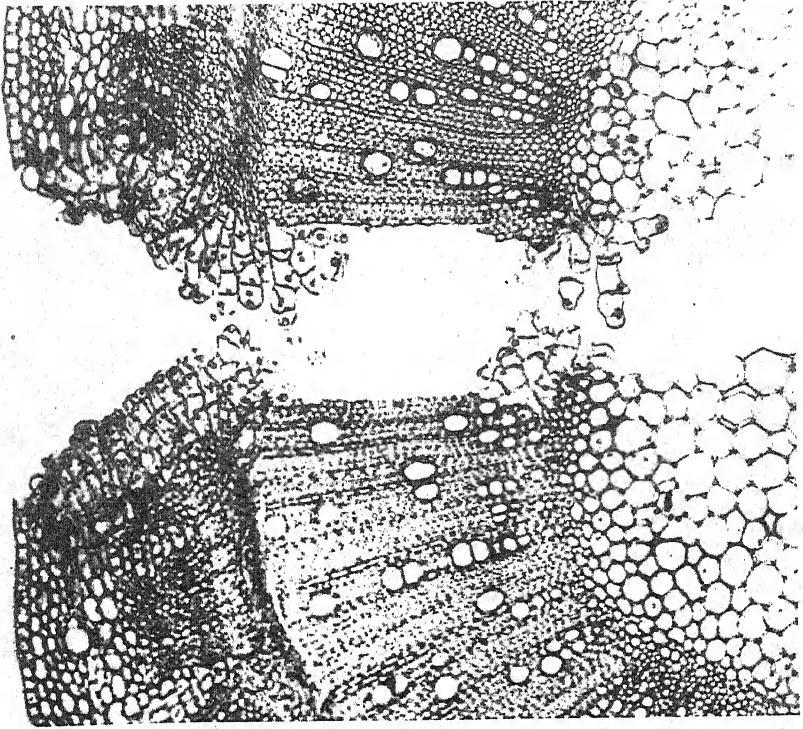


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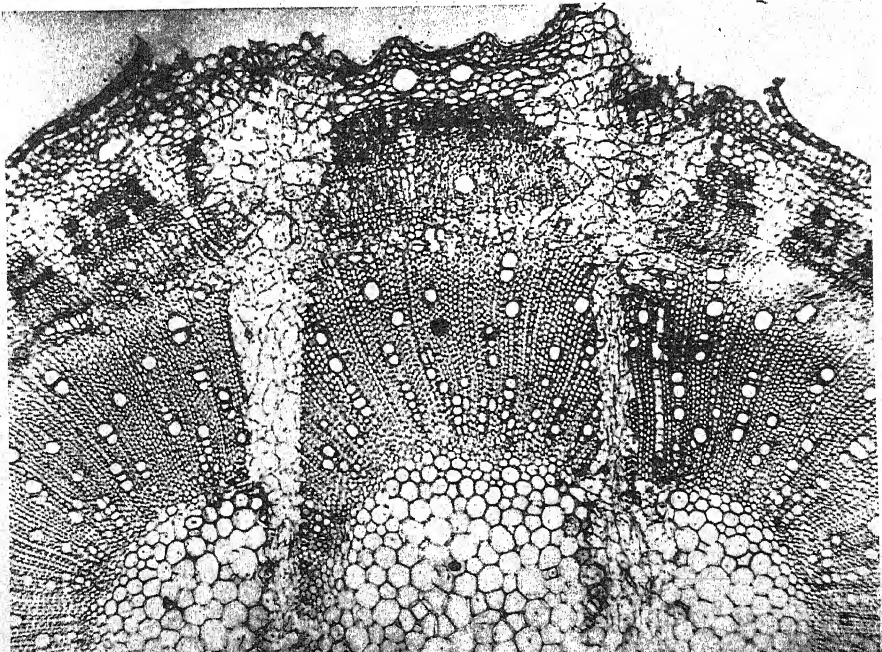


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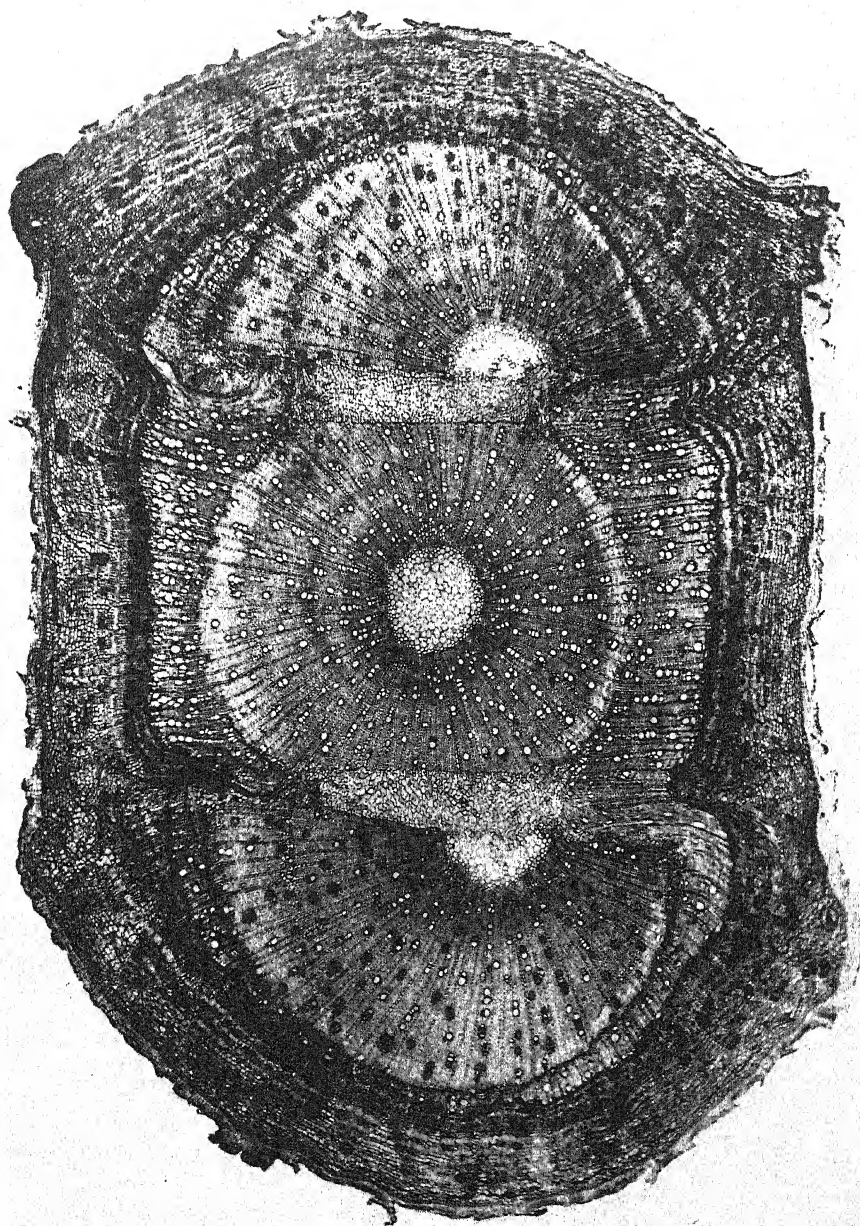


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# The Effect of Soaking in Water on the 'Seeds' of *Dactylis glomerata* L.

BY

H. G. CHIPPINDALE, M.Sc.

With one Diagram in the Text.

AMONG the grasses regarded as desirable by agriculturists, and commonly obtainable from commercial seedsmen, *Dactylis glomerata* L. (cocksfoot) is characterized by having 'seeds' (i.e. caryopses with attached pales) which are slow and capricious in germination. Stapledon, Davies, and Beddows (8) found that seedlings of this grass required 13 days before appearing above the surface of the soil in comparison with a requirement of 7 days for *Lolium perenne* and 11 days for *Alopecurus pratensis* under the same conditions. Seedlings from a single sowing of cocksfoot frequently appear in successive batches separated from one another by considerable intervals of time, germination being completed only after several weeks, this occurrence being particularly frequent in the case of seeds sown under glasshouse conditions. The results given in the present paper offer an explanation of this peculiarity and a means for its elimination.

Zade (10) studied the germination of cocksfoot in some detail and found that a considerable variation in behaviour occurred among different strains. With most, the ripe seeds when freshly harvested were non-germinative and required a period of about 3 months to pass out of this condition; repeated alternation of soaking in water and drying accelerated this transition. Subsequently, good germination occurred when the seeds were subjected to a variable temperature but, except in the case of certain strains and of seeds more than 2 years old, germination was exceedingly poor with a temperature relatively constant, such as usually obtains (in Zade's opinion) under field conditions. This defect could be overcome by wounding the caryopses (for which purpose mechanical threshing was effective) but not by removing the pales from them nor by soaking in water. Tincker (9), however, found the latter treatment to increase the germination of cocksfoot and other grasses under soil conditions, but the degree of acceleration was not shown by his figures, and the age of

the material was left unstated. A slight acceleration of germination from soaking in water was also obtained with cocksfoot seeds by Kinzel (5).

The marked reaction of the seeds of this grass to a variable temperature found by Zade was in agreement with earlier work (6 and 7), and more recently Harrington (2) also observed this phenomenon. In the orthodox practice of seed testing stations it is usual to subject seeds of cocksfoot to an alternating temperature during germination.

#### METHODS.

The seeds used in the present experiments were all of sufficient age to have passed out of the non-germinative condition described by Zade; they had all been mechanically threshed and included samples of both 'commercial' and indigenous cocksfoot. For the purpose of germination the seeds were set out on discs of filter-paper saturated with distilled water and placed in closed Petri dishes. In almost every case the figures in the ensuing tables represent averages of three separate lots each of 100 individuals; the differences due to the various treatments to which the seeds were subjected are sufficiently large to render statistical treatment unnecessary. Samples of cocksfoot seed are made up of single seeds of very various sizes and of clusters of two or three seeds which remain attached and represent the terminal flowers of the spikelet; except where otherwise stated, these elements have been taken at random for the present work and each has been regarded as a single individual.

In the experiments involving the soaking of seeds, the latter were floated on the surface of distilled water in the proportion of 25 c.c. of water to 1 gm. of seed; wide dishes were employed, the conditions of aeration being good. The soaking was maintained for 17 hours at 20° C., followed in most cases by 24 hours drying in the open air at about 14° C.

#### RESULTS.

The figures in Table I clearly show the difference in germination obtaining between seeds kept at a temperature approximately constant at 19° C., and similar seeds subjected for daily periods of 5 hours to a lower temperature (9° C.). This beneficial effect of a fluctuating temperature has been observed to be general in the course of the present work, but the susceptibility of several samples is different. A distinct indication has been obtained that this property depends upon the conditions under which the seed has matured, that grown in an environment favourable to this process reacting less markedly to a varied temperature. It is clear that the effects of the varied temperature would have been more considerable had the seeds been placed during the shorter daily period at a temperature higher than 19° C., but such a procedure (which was the one adopted by

earlier workers) does not differentiate between an acceleration due to a higher temperature and that due to the fluctuation. As they stand, however, it is doubtful whether the differences shown in Table I are sufficiently great to be of practical importance in the field, but in consideration of the very much lower germination which occurs when the seeds are covered with soil (cf. Table II below) it is probable that the detrimental effect of a relatively constant temperature is occasionally of importance in agricultural practice.

TABLE I.

*Percentage Germination of Cocksfoot Seeds at a Constant and at a Varied Temperature.*

	Temperature.	After—		
		11 days.	14 days.	30 days.
Commercial cocksfoot	constant	29	62	90
	varied	47	68	84
Indigenous cocksfoot Bc S 35; 15 months old	constant	1	22	70
	varied	13	32	84
Indigenous cocksfoot Bc 1163; 4 months old	constant	8	33	79
	varied	16	39	82

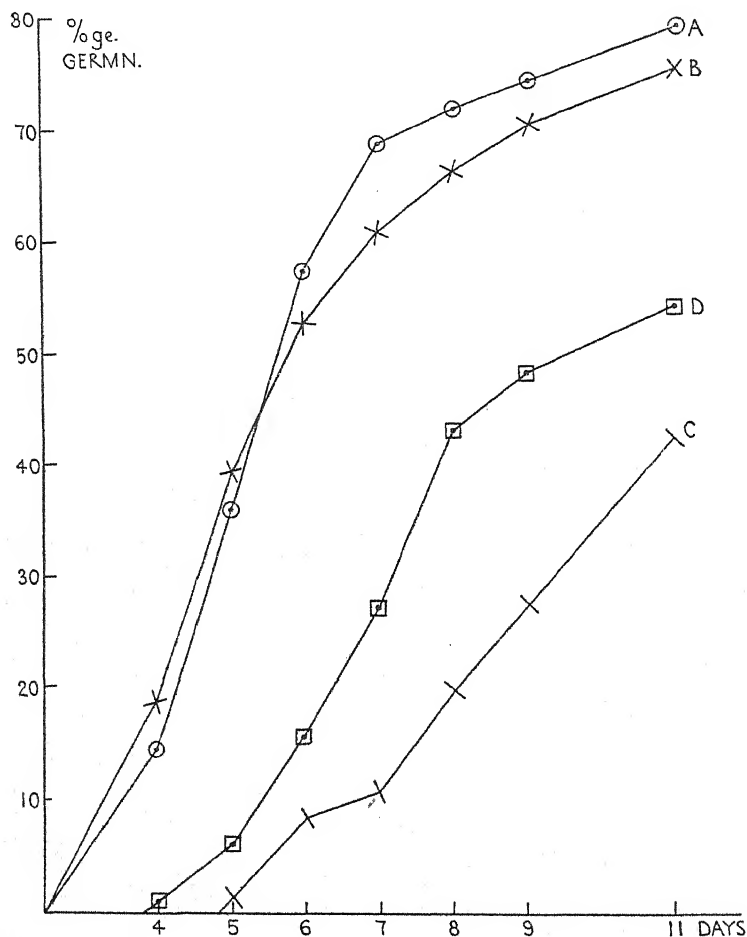
It has been found, however, that the germination of cocksfoot seeds subjected to either a constant or a variable temperature, can be very decidedly accelerated by the simple treatment of soaking the seeds in water and afterwards allowing them to become air-dry before sowing. This effect is illustrated in the diagram on p. 844, which has been derived from indigenous seeds germinated at a temperature of 19° C., 14 months from time of harvesting; in this case large single seeds only were employed so that the curves are directly comparable with those for naked caryopses in the same figure. An acceleration of three days is given, but more considerable benefits are shown in Table II, the figures of which are representative respectively of commercial seed; commercial seed grown in Britain; indigenous seed 4 months, 20 months, and 6 years old; and 15-month-old indigenous seed germinated in sterilized soil.

In the last case it is noteworthy that the germination of the control seeds is still much inferior to that of the soaked seeds 5 weeks from the time of sowing; with seeds on filter pads on the contrary, the final differences are small. The experiment with soil was the only one of those scheduled in the table in which the seeds were not soaked for 17 hours and air-dried for 24 hours. The results given in Table III, however, show that there is nothing specific in the time of drying or in the degree of dryness of the seed; an extended period of soaking is advisable since, as described later, the seeds are not readily wetted and absorption of water is slow.

It should be noticed in Table II that the effect of soaking is relatively

more pronounced in the case of those samples of seed which show the lowest total germination.

This acceleration of germination following soaking and drying is in accordance with the results of Tincker and Kinzel, but contrasts with those



Germination of indigenous cocksfoot (Bc S 36, 14 months old) at 19° C. A. Unsoaked caryopses. B. Soaked caryopses. C. Unsoaked 'seeds' (caryopses with pales). D. Soaked 'seeds' (caryopses with pales).

of Zade (which were obtained with seeds threshed by hand). It has, however, been observed without any exception in a considerable number of samples of cocksfoot seed, both of commercial and indigenous origin, so that the phenomenon can be considered of general occurrence with the seeds of this grass available in Britain.

In considering how this acceleration of germination by soaking is

brought about, one is unfortunately confined almost entirely to inferential methods. Seeds of cocksfoot normally contain about 10 per cent. (of the gross wet weight) of water; after soaking and drying as described above, the moisture-content varies considerably but remains higher than in the untreated seeds by 2 to 7 per cent. That this difference, however, is with-

TABLE II.

*Percentage Germination of Soaked and Unsoaked Cocksfoot Seeds.*

		Temperature.	8 days.	After— 14 days.	32 days.
Commercial cocksfoot	untreated seeds	13°-17° C.	47	69	88.5
	soaked "		74	90	91
Indigenous cocksfoot Bc 1163, 4 months old	untreated seeds	"	9 days. 26	16 days. 71	
	soaked "		37	73	
Commercial cocksfoot Grown in Britain	untreated seeds	"	10 days. 19	13 days. 42	28 days. 66
	soaked "		58	66	73
Indigenous cocksfoot Bc 1163, 20 months old	untreated seeds	"	11	27	74
	soaked "		61	75	82
Indigenous cocksfoot Bc 1163, 6 years old	untreated seeds	"	1	6	31
	soaked "		13	25	42
Indigenous cocksfoot Bc 1163, 15 months old (Sown in sterilized soil.)	untreated seeds	12°-17° C.	11 days. 29	13 days. 39	36 days. 41
	soaked "		57	61	64

TABLE III.

*Percentage Germination of Indigenous Cocksfoot Seeds Dried for Different Periods after soaking 17 hours in Distilled Water.*

		Weight per 1,000 seeds.	10 days.	After— 13 days.	32 days.
Bc 1163, 16 months old Temp. = 12°-17° C.	untreated	1.1014 gm.	29	55	77
	soaked and dried				
	1 hour	1.7833 "	54	69	84
	1½ hours	1.4313 "	73	80	89
	2½ "	1.2632 "	72	79	84
	4 "	1.2035 "	72	78	83
Bc 1314, 2 years old Temp. = 19° C.	24 "	1.0774 "	72	79	86
	untreated		46	66	77
	soaked and dried 9 months		62	73	84

out appreciable effect on germination when adequate water is present in the substrate is shown by the figures in Table III and by the fact that the benefits of soaking are undiminished by drying the seeds over concentrated sulphuric acid to a lower moisture-content than that of the untreated seeds. Also, seeds which have been soaked germinate earlier than the controls



when both are placed on the surface of distilled water, so that the effect is not related primarily to a deficiency of moisture in the substrate, although it is obvious that where such a deficiency obtains the greater moisture-content of the soaked seeds will be an advantage. If, however, the pales which normally enclose the caryopses are removed (without injury to the latter) germination is very greatly accelerated and the effect of soaking on such caryopses is negligibly small (cf. Diagram on p. 844 which illustrates both these phenomena). It is, therefore, clear that the acceleration of germination, due to the latter treatment, in complete seeds is brought about by some action of that treatment on the pales.

Harrington and Crocker (3) found that the germination of *Sorghum halepense* was inhibited by the pales mechanically resisting the swelling of the caryopsis and hence preventing the entrance of water. A simple method which can be used as a criterion of this behaviour was found to be boiling the seeds in water, which caused the constricted caryopses to burst the pales. The results of applying this test to cocksfoot have been entirely negative, and the small size of the caryopses relative to that of the pales removes all possibility of the phenomenon observed by Harrington.

Experiments into the possibility of substances inimical to germination occurring in the pales have also given entirely negative results.

In Table IV is shown the rate of entrance of water into soaked (and dried as above) and untreated seeds of cocksfoot, and a differentiation in favour of the former is evident. The figures in the table were obtained from seeds enclosed in wire-gauze and immersed in distilled water; they represent, therefore, absorption under optimum conditions, and greater differences might be expected with a diminished supply of water. The different behaviour of soaked and unsoaked seeds is shown immediately on immersion in water; the surface of the former is at once wetted and the pales rapidly acquire a water-soaked appearance, whereas the unsoaked seeds repel water and remain unwetted for some time. With seeds placed on wet filter-pads in Petri dishes this phenomenon manifests itself in the occurrence of dew only on the unsoaked seeds, where it persists usually for at least 10 days, but is eventually absorbed. Both these symptoms of differentiation are still to be observed in seeds, which have been air-dried for 9 months. No evidence has been obtained, however, that it is due to structural or chemical alteration from soaking (the separation of the caryopsis from the pales on absorption of water as described for *L. perenne* by Brown (1), does not occur, with cocksfoot), and the explanation would seem to depend on surface-tension phenomena, most probably on the cracking or removal of a surface layer of wax or fat.

It is clear that we have here a factor favouring the rate of germination of soaked seeds comparative to that of the untreated, and which probably would account in itself for the results given above. There remains, how-

ever, the further possibility that the permeability of the pales to gases is affected by soaking in water.

That the pales hinder the respiration of the seeds is shown by the marked acceleration of germination which follows their removal, or the removal merely of a narrow strip from the dorsal pales, and which occurs also in seeds surrounded by an atmosphere abnormally rich in oxygen.

TABLE IV.

*Absorption of Water by Seeds of Cocksfoot per gram of Dry Matter.*  
(Temperature 22° C.).

	Unsoaked seeds.	Soaked seeds.
After 3 hours	0.7730 grm.	1.2961 grm.
" 5 "	0.8192 "	1.2116 "
" 24 "	1.0712 "	1.2093 "
Percentage moisture (of wet weight) after 24 hours	51.7	54.8

Further, the movement of gases directly through the pales would seem essential to germination since covering the dorsal surface of the pales with vaseline, if only overlying the embryo, is sufficient to prevent it occurring for a considerable time (viz. about 21 days). It is unfortunate, therefore, that it is impossible to determine directly the relative permeability of the soaked and unsoaked pales to gases, but indirect evidence does not suggest that there is any very considerable difference between them. Seeds of both kinds which have been exposed to fumes of ammonia show no differentiation in viability, and although the rate of respiration in soaked seeds exceeds that of the unsoaked for the first 24 hours that the seeds have been under conditions suitable for germination, the reverse is the case for the second day.

#### CONCLUSION.

The uniformly beneficial results which have followed the soaking of cocksfoot seeds in the manner described above, their independence of precision in method, and the absence of evidence that under any circumstance such treatment is deleterious, suggest the desirability of making this operation a routine practice in laboratory experiments with the seeds of this grass, and especially should the viability of the sample be low. In the case of germination-tests, however, any assistance given to inferior samples is objectionable since a differentiation of such is sought after.

In considering the significance of the above results to field practice it must be remembered that they have been obtained at temperatures higher than are usual in the open ground, and that any time interval between the

germination of soaked and untreated seeds will tend to be greater at lower temperatures. Frequently, however, the seeds must be subject to soaking and drying by natural means. With seed sown under glass this does not apply, and the conditions unfavourable to germination, viz. a relatively constant temperature and a shortage of free water, are extremely liable to occur ; soaking of the seeds prior to sowing would therefore seem advisable in every case.

Evidence has been obtained that the phenomenon described in the present paper is not of general occurrence amongst the grasses, and it is clear from the results of earlier workers that it does not invariably occur with all strains of cocksfoot. It is manifest, therefore, how undesirable it is that any generalization should be made regarding the beneficial effects of soaking on the seeds of plants generically or even specifically different. In many cases it is probable that the mechanisms involved are each peculiar to a particular species, or even to a particular strain.

#### SUMMARY.

1. Seeds of cocksfoot (*Dactylis glomerata* L.) germinate better at a varied than at a constant temperature, particularly if matured under somewhat unfavourable conditions.
2. Under either temperature condition, germination is considerably accelerated if the seeds have, at any time previous, been soaked in water and subsequently dried.
3. This effect is due to the seeds so treated absorbing water more rapidly than the normal seeds, the pales of which are at first impermeable.

I wish to express my indebtedness to Professor R. G. Stapledon under whose auspices the work has been performed.

WELSH PLANT BREEDING STATION,  
ABERYSTWYTH.

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# A Contribution to Our Knowledge of *Woronina polycystis* Cornu.

BY

W. R. IVIMEY COOK

AND

W. H. NICHOLSON.

With sixteen Figures in the Text.

THERE seems very little doubt that *Woronina polycystis* is quite a common parasite of the Saprolegniales, although actual records of its occurrence are comparatively rare. This is due chiefly to the fact that very few mycologists have studied the Water Moulds in any detail, and consequently records of its appearance are lacking from most mycological lists. *W. polycystis* was first observed by Pringsheim (6) in 1858, who regarded it as the antheridia of the *Saprolegnia* on which it was found. Later, Cornu (1) in 1872 made a more critical study of the organism, recognized that it was a parasite, and interpreted the stages which he found in a more or less complete description. In 1882 Fischer (3) made a further study of the life-history, and confirmed in most points Cornu's records of ten years earlier. From that time, with the exception of brief records (2, 4, 5) of its occurrence, little work has been done on the organism.

In a recent survey of the Saprolegniales carried out in the neighbourhood of Bristol, *W. polycystis* appeared several times in the different places where samples of water had been collected, and it was decided to make a further study of the organism in the hope of clearing up some of the points that still remain unsettled.

The parasite attacks the hyphae of a number of species of the Saprolegniales, but owing to the distortion and damage which it causes to the host, and the fact that it generally prevents the host forming sex organs, it is difficult to identify the particular species of Water Mould which is subjected to the attack. In the Bristol district the parasite has been obtained from the River Avon at Saltford, in a stream near Winterbourne

and in another stream near Chew Magna, and from these localities five species of *Saprolegnia* and ten species of *Achlya* have been isolated, so that it is difficult to say definitely in which the parasite occurs. It seems likely, however, from the appearance of the host filaments, that many different species are parasitized, and that *W. polycystis* is able to infect any species of *Saprolegnia* or *Achlya*. Owing to the conditions under which the plants were grown, it was possible to study the growth of the parasite concurrently with that of the host, since both were grown together in water from the original locality, using hemp seed as a substratum. It was also found that it was possible to keep the parasite alive by growing the host on maize agar, though owing to the fact that no reproductive organs were formed by the host, it tended to disappear in time, and unless fresh *Saprolegnia* plants were supplied the parasite also disappeared after a few months. Since, however, the parasite was growing under comparatively natural conditions it was found easy to observe the discharge and development of the zoospores, and in this matter especially fresh information was obtained.

It is convenient to start a consideration of the life-history with the plasmodium, since this, at any rate in the later stages of its development, is a very noticeable structure in the cytoplasm of the host. In a very young stage it is difficult to separate the plasmodium from the cytoplasm of the host, but it was found that by treatment with methylene blue it was possible to obtain a difference in the staining reactions between the two structures and that under such treatment the plasmodium stained up more densely than the host cytoplasm (Fig. 11). These young plasmodia are composed of very finely granular cytoplasm and are at first uninucleated and devoid of any limiting membrane. They are irregular in shape, but tend to become roughly spherical in outline, and as far as has been observed these plasmodia are incapable of movement. They grow at the expense of the host cytoplasm becoming multinucleated, and eventually completely filling the host cell (Fig. 1). During the early stages of their development the host filament develops septa, with the result that the parasite may be isolated from the rest of the filament, but generally a small part of the plasmodium becomes separated in this process and passes through into the lower part of the hypha, where it also develops until the host, stimulated by its activity, develops another septum when the same process is repeated. In this way a series of plasmodia are formed in succession from the apex of the filament, each one being younger than the one nearer the growing end of the host hypha.

The parasite feeds mainly upon the globules of oil present in the host cells. The presence of these droplets was demonstrated by staining the hyphae with osmic acid and Sudan III, and in every case the same reaction was obtained for the host cell and for the parasite during its younger stages. Mature plasmodia and sporangia do not show this reaction, since in them

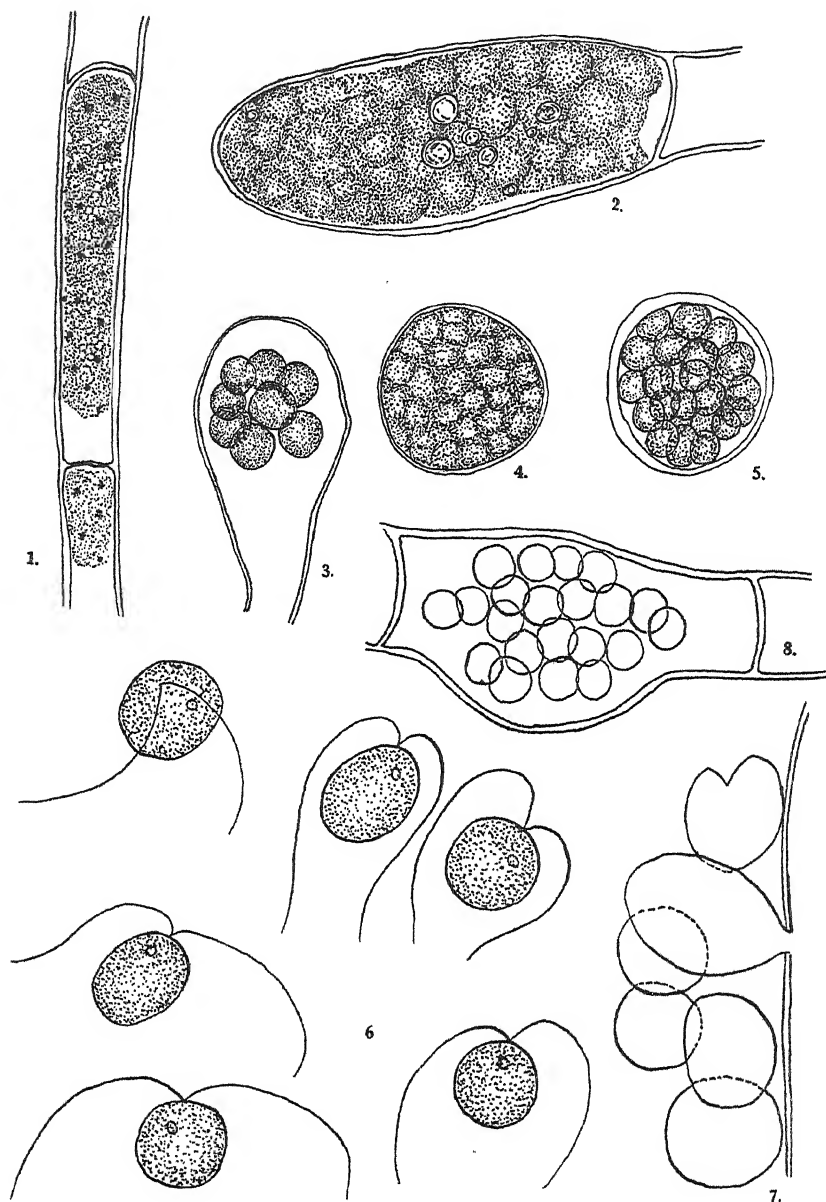
the oil has become converted into proteins, as was demonstrated by treatment with Millon's Reagent. From these microchemical tests it seems, therefore, that the food of the parasite is glycogen and similar oils which are found in the hyphae of the Saprolegniales, and it is interesting to note that the only other species of *Woronina* known occurs in *Vaucheria* in which oils are also present. Tests with iodine show that no starch is present in the parasite.

The mature parasite consists of a naked plasmodium which fills the host cell, in fact, the host cell may become swollen as a result of parasitism, and this is particularly true of the apex of the filament. The plasmodia vary greatly in size, but average  $282\mu$  long by  $45\mu$  wide, although plasmodia up to  $476\mu$  by  $60\mu$  are sometimes found. During the development of the plasmodium, the nuclei divide up into a large number, and when the plasmodium is mature these nuclei become equally spaced throughout the protoplasm. The latter then becomes furrowed (Fig. 2), and in these furrows cellulose is laid down and the whole is divided into a number of sporangia (Fig. 3). At the same time the whole plasmodium becomes surrounded by a cellulose wall.

The size and arrangement of the sporangia varies greatly. Very frequently they form a mass rather towards the centre of the cell, and this is particularly true of sporangia formed in swollen apical cells. In other cells they may spread out more evenly so as to completely fill the host cell. In general, there seems to be a slight contraction of the protoplasm of the plasmodium at the time of sporangium formation. The sporangia measure from  $12$  to  $20\mu$  in diameter with an average of  $16.7\mu$ . They are roughly spherical in shape when containing zoospores, but after their liberation they may revert to a more hexagonal appearance. In this condition they give the host cell the appearance of being a dictyosporangium, and it is not unlikely that in some instances they have been used as evidence of the occurrence of dictyosporangia in certain species of *Achlya*. The wall of the sporangium is very thin, smooth, and shows no markings or sculpturing. It turns faintly violet with chlorzinc iodide, indicating that it is composed of cellulose, this reaction being similar to that given by the host filaments. The contents of the mature sporangium eventually divides up into a number of uninucleated parts separated from one another by a very delicate membrane (Fig. 5). This membrane does not give the same reaction with chlorzinc iodide, and it is thought that it represents merely a thickening of the protoplasm and is not a true wall. When mature, the contents of the zoosporangium are made up of a number of tiny spherical zoospores.

Meanwhile those sporangia which are in contact with the wall of the hypha form short processes which perforate the wall, while the ones towards the centre of the mass remain without any means of discharge





The drawings were made with a camera lucida at table level, the magnifications are given after the description of each figure.

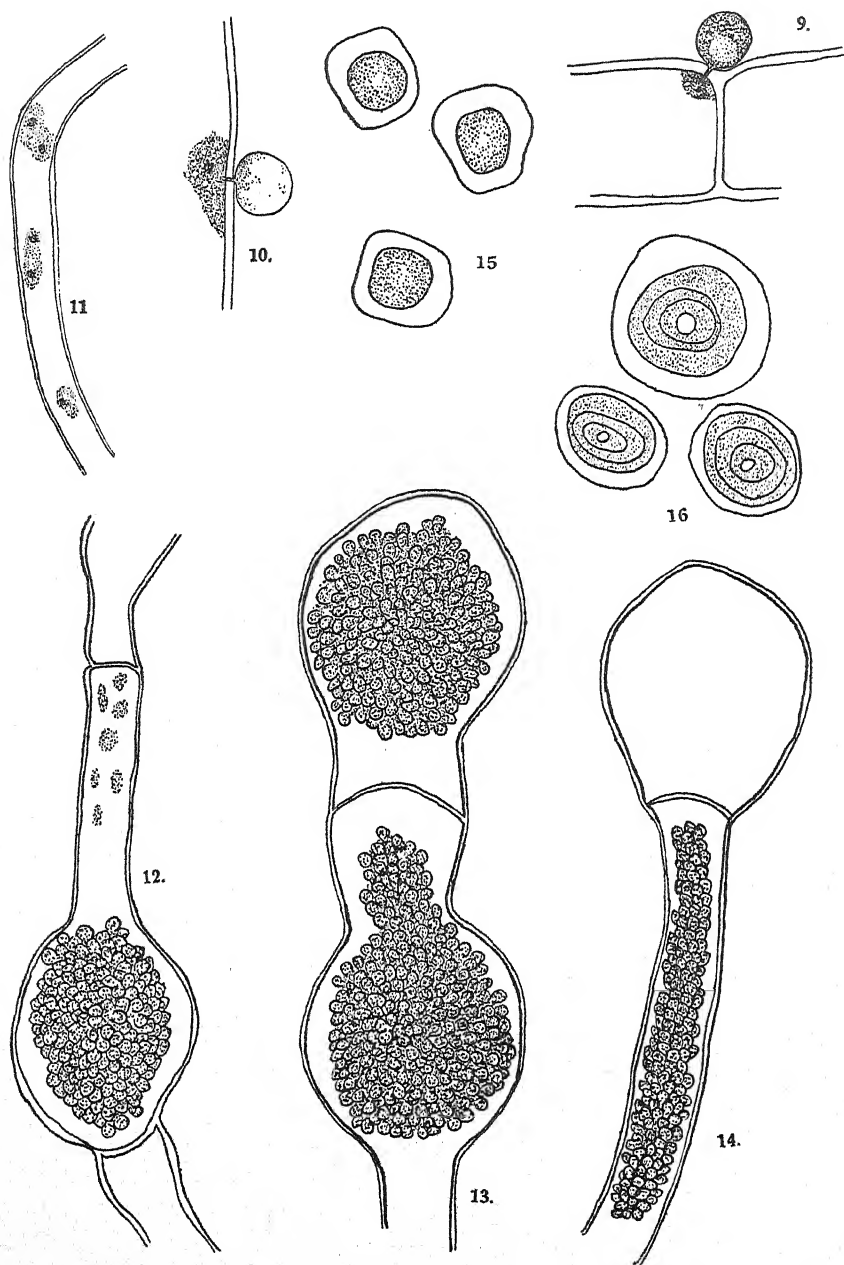
FIGS. 1-8. FIG. 1. Plasmodium almost filling the host cell, and a younger one separated by a septum.  $\times 380$ . FIG. 2. Plasmodium showing furrowing prior to the formation of zoosporangia.  $\times 620$ . FIG. 3. Zoosporangia lying freely in the host filament.  $\times 580$ . FIG. 4. Zoosporangium, showing the division of the cytoplasm into zoospores.  $\times 1280$ . FIG. 5. Zoosporangium in which the zoospores have been delimited.  $\times 1280$ . FIG. 6. Zoospores after liberation,  $\times 1280$ . FIG. 7. Zoospore showing the path of movement.  $\times 1280$ . FIG. 8. Zoosporangium in which the zoospores have been delimited.  $\times 1280$ .

(Fig. 7). Finally, the zoospores begin to escape from the peripheral zoosporangia through the tubes, and as this continues the wall between the discharging zoosporangium and those immediately adjoining it breaks down, presumably owing to the pressure exerted by the full sporangia against the empty one. The zoospores pass through the opening into the empty zoosporangium and thence to the exterior by the same opening. This method of discharge explains why it is so frequently observed that discharge only takes place from a few points on the hypha. A few of the zoosporangia generally discharge their contents into the host filament, and these zoospores can be observed swimming about seeking a way out. This is usually provided by the enlarged opening around which a perforation has been made by an exit tube. It takes about three-quarters of an hour for the whole of the contents of a large cluster of zoosporangia to escape, although the greater part emerges during the first ten minutes.

The zoospores swim away immediately after discharge, being already provided with flagella. Each zoospore (Fig. 6) is spherical, surrounded by a very delicate membrane, and possesses two apical flagella which are generally rather more than twice the length of the zoospore and are usually directed backwards as the zoospore swims. These zoospores are fairly constant in size, being  $3.5-4\ \mu$  in diameter (average  $3.8\ \mu$ ). They can remain active for several hours. After liberation they swim actively until they come into contact with a suitable hypha, selecting as a rule the tip or some place near it. Here they become attached by their apical end and a very fine infection hypha is pushed through the wall (Fig. 8). It seems possible that the flagella themselves assist in this process. Penetration is effected and the contents of the zoospore pass into the host tissue forming a tiny amoeboid body on the inside of the wall (Fig. 9). The amoeboid body then becomes separated from the infection hypha and may be washed round the cell by the action of the cytoplasm. It finally assumes a more central position where it develops into a fresh plasmodium, there being no resting stage in the development of the zoospores.

When the parasite has become old and the host tissue exhausted, a second type of reproduction may be resorted to, which is specially adapted to adverse conditions, by the formation of resting bodies, termed cystosori (Figs. 12 and 13). Each originates from a single plasmodium which divides up into a number of uninucleated masses, each of which is surrounded by a thick wall. Microchemical tests prove that this wall is composed of cellulin. These cystosori are very variable in shape, depending largely upon the shape of the plasmodium from which they have originated. Usually they occur terminally or laterally in large swellings of the host

showing the flagella directed backwards from the anterior end.  $\times 3200$ . FIG. 7. Empty zoosporangia showing the exit tube through which the zoospores have escaped.  $\times 920$ . FIG. 8. Group of empty zoosporangia in host filament.  $\times 580$ .



The drawings were made with a camera lucida at table level, the magnifications are given after the description of each figure.

FIGS. 9-16. FIG. 9. Early stage in infection by a zoospore showing the infection hypha penetrating the host wall.  $\times 1300$ . FIG. 10. Late stage in infection by a zoospore showing the almost

tissue, though occasionally intercalary swellings may also occur. The spherical cystosori vary from  $50$  to  $77\mu$  with an average of  $70.5\mu$  in diameter, while elongated ones up to  $308\mu$  long have been found. Each cystosorus is made up of a large number of thick-walled spores, but there is no common membrane around them.

The spores (Fig. 14) themselves are oval or spherical and vary in size from  $5.5$  to  $8.6\mu$  in diameter (average  $7.8\mu$ ). The wall is very thick and the contents consist of a small quantity of protoplasm, a single nucleus and a reserve of oil or fatty substances, the latter presumably serving as a food reserve.

In all the cases observed, the cystosori remain within the host cells, but it seems likely that in nature the whole cystosorus may be liberated by the disorganization of the dead host tissue.

The actual germination of these resting spores has not been observed, although many empty spores have been found. From their appearance it is obvious that the zoospore emerges through a small opening in the wall of the spore (Fig. 15). Active zoospores were seen swimming in cultures containing these empty cystosori, and, since as far as could be found no zoosporangia were present in the cultures at the time, it is probable that they came from these resting spores. They settled down and infected the hyphae in a way entirely similar to the zoospores already described. Since it was not possible to follow them from the time of their escape from the resting spores, there is no evidence that any conjugation had taken place, but from their size this seems unlikely. Unless conjugation does occur at this stage, no evidence has been found to indicate that any conjugation or sexual process occurs in this species.

As to the conditions most favourable for the development of the resting spores, it was found that a sudden change from a low temperature of  $4-5^{\circ}\text{C}$ . to one of  $20^{\circ}\text{C}$ . was the most suitable, provided that a supply of actively growing *Saprolegnia* filaments was present in the culture. Little attention was paid to the selection of the species of *Saprolegnia* used for this purpose, although it is known that *S. ferax* and *A. de Baryana* can be successfully parasitized, but, as has already been stated, there is evidence that *W. polycystis* can attack any species of these two genera.

#### DISCUSSION.

It is not proposed here to enter into the complex question of the affinities of *W. polycystis* with the other genera which have been placed by

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empty zoospore and the formation of an amoeboid body within the host.  $\times 1300$ . FIG. 11. Young plasmodia shortly after the separation of the amoebae from the infection hyphae.  $\times 380$ . FIGS. 12-13. Mature cystosorus lying in the apical segment of a swollen hypha.  $\times 580$ . FIG. 14. Mature cystosorus showing the elongated shape sometimes adopted.  $\times 580$ . FIG. 15. Some resting spores showing the thickness of the wall.  $\times 2400$ . FIG. 16. Some spores after the escape of the spore, showing the small hole by which it escaped.  $\times 3000$ .

von Minden (5) in the Woroninaceae. Our knowledge of the other genera is still scanty, and much of it is based on early work which has not been confirmed by later observers. If it is true that there is no sexual reproduction in *W. polycystis* and that sexual reproduction always occurs in the genus *Olpidiopsis*, there might be an advantage in separating these genera more widely than is at present done. In the genus *Pseudolpidinum* are included those species of *Olpidiopsis* in which a companion cell does not occur, or has not been so far observed, but few of the species have been fully studied and eventually it may be found more satisfactory to include them all in the genus *Olpidiopsis*. The genus *Rozella* may probably be related to *Woronina*, but it is doubtful whether the resting spores described by Cornu really belong to this genus. Should it be found that these are structures belonging to another fungus, the relation between the two genera would be closer than appears at present. So far this genus has not been found in the Water Moulds collected from the Bristol district. The little known *Pyrrhosorus marinus* described by Juel may also belong to this family.

Probably the most important point which the present investigation has brought to light is the fact that the zoospores have two apical, and not lateral, flagella. There can be no doubt of this since they were observed many times by independent observers, all of whom were agreed that they were apical, though directed backwards in motion. This is not in accordance with what has been observed in the other genera, where the zoospores have two lateral flagella. Some authorities, basing fundamental importance to the position of attachment of flagella, would therefore regard this as sufficient evidence for separating the genus *Woronina* from the remaining genera, but it seems more desirable to retain the genera as they are until more critical work has been done on these other genera and the position of their flagella verified.

It may be mentioned, however, that these zoospores of *W. polycystis* are quite distinct from the pyriform, unflagellated zoospores or swarm spores found in the Plasmodiophorales, and constitute another point which seems to preclude a close relationship between the two groups.

#### SUMMARY.

1. The life-history of *Woronina polycystis* has been investigated. The development of the plasmodia and zoosporangia has been followed and the discharge of the zoospores observed.
2. The zoospores possess two apical flagella which are directed backwards during swimming; they are about twice the length of the zoospores.
3. The development of the cystosorus has been followed. The escape

of the motile spores has not been seen, but it has been found that they escape by a small perforation in the wall without any disintegration of the cystosorus.

4. Infection by the zoospores has been followed from the time of their escape, and no evidence of any fusion or resting period has been found.

5. The systematic position of *W. polycystis* is briefly reviewed.

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# The Reproduction of *Plantago Coronopus*: An Example of Morphological and Biological Seed Dimorphism.

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With three Figures in the Text.

DURING the course of an investigation into the ecological relationships of *Plantago Coronopus* a considerable amount of attention has been given to the reproductive capacity of the species. The plants are propagated both by seed and by vegetative means, and the following is an account of the structure of the fruit and seed and of the germination of the latter. At the same time observations on the vegetative phases and longevity of the plant are recorded.

The axillary spikes which form the inflorescences of this plant vary in position and size with the leaf form. When the leaf rosette is flat the inflorescence stalks are in the same plane as the leaves, and only the flower-bearing part of each axis is raised above the general level. Where the leaves are more erect the spikes are nearly vertical, and in some of the largest plants the inflorescences are sometimes as much as 40 cm. long, while in *P. Coronopus f. pygmaea* they are frequently less than 1 cm. in length.

Each flower is subtended by a bract and consists of four green persistent sepals, and of these, the two on the posterior side are conspicuously keeled and hairy. Alternating with the sepals are four whitish transparent petals. Typically the flowers are hermaphrodite, having four stamens with long filaments, and large yellow versatile anthers and a syncarpous ovary surmounted by a long hairy simple style.

A transverse section of the ovary in the middle region shows that it is bi-carpellary, each carpel having two ovules separated from one another by a large mass of tissue which frequently extends to the ovary wall but is not fused with it (cf. Hooker 'Flora Australensis', Vol. V, p. 139). More especially in the young condition this tissue forms a cup round each ovule, completely separating it from its neighbours (Fig. 1 A and B).

The ovary gives rise to a capsular fruit which dehisces by an encircling



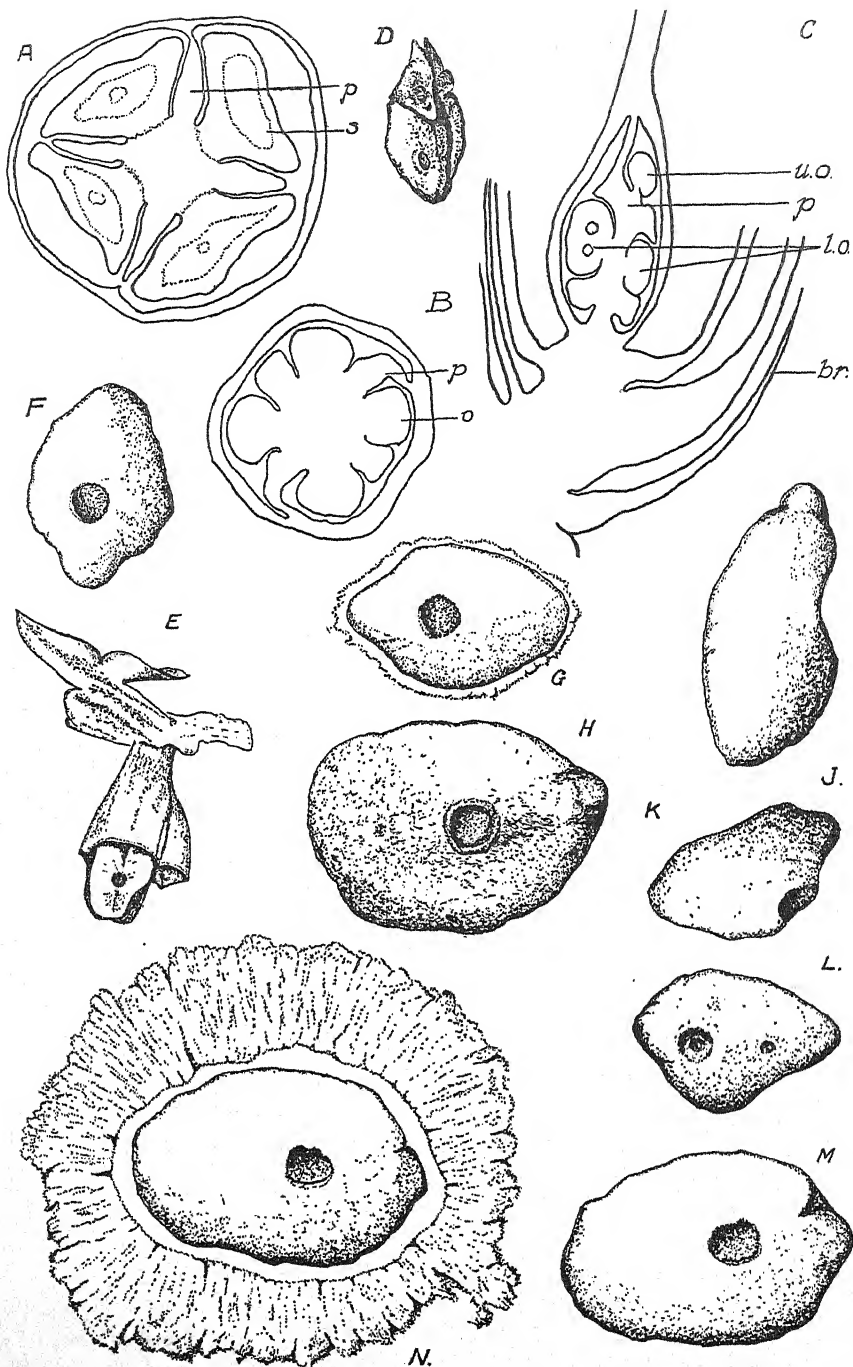


FIG. 1. A. Transverse section of an almost mature capsule with two well-developed seeds (*s*) in each loculus. The placenta (*p*) has already broken away from the ovary wall on one side.  $\times 33$ . B. Transverse section of a young ovary showing four ovules, two in each loculus.  $\times 64$ . C. Longi-

transverse split thus forming a lid which separates generally with the placental axis attached. In this way four seeds, or less frequently some smaller number, are released. These seeds, which are rather more than 1 mm. long and about 0.7 mm. wide, are oval in shape and have the hilum on the inner face. The outer side is convex, with the result that the seeds almost completely fill the capsule. This is the condition hitherto described, but this picture is incomplete as, in addition to these seeds which are all similarly placed in relation to the long axis of the capsule, there is another seed above the others which has been overlooked. This seed is so placed that it is usually shed with the capsule lid and kept in position by the placental core (Fig. 1, D and E). This seed is found constantly in both large and small forms of the species and as a consequence the capsule contains frequently five or four seeds, the latter condition being due to the absence of one of the lower seeds and not to the abortion of the upper seed, which very rarely occurs. It has been stated frequently that the ovary of *P. Coronopus* contains four seeds or less by abortion. As far as the material examined is concerned the following figures show that the four-seeded condition is *not* the most common, and that abortion is not frequent. Some 670 capsules were examined, and of these 73 per cent. contained five seeds each, while only 19 per cent. had four seeds. Of the remaining 8 per cent. there were 5 per cent. of the capsules with three seeds each, and 2 per cent. with two seeds. The remaining 1 per cent. were capsules which either had more than five seeds each and those which had but a single seed. Thus not more than 27 per cent. of the capsules showed abortion of any of the ovules.

A longitudinal section of the capsule confirms the position of the upper seed and shows that the placenta is axile though somewhat out of the median longitudinal plane due to the presence of this upper seed in one loculus only. This seed is produced, not in a separate loculus, but in the upper part of the anterior loculus of the ovary between the two lower ovules and separated from them by an outgrowth of tissue similar to that which separates them from one another (Fig. 1 C).

The seed at the top of the fruit has its long axis at an angle to that of the capsule, with the result that not only is the seed much smaller than the others, but it is also of a different shape (Fig. 1, H, J, K, L).

tudinal section of a young ovary of a typical plant, showing two lower ovules (L.o.) and the upper ovule (u.o.) attached to the axile placenta (p). × 33. D. Placental tissue removed from the capsule lid to show the position of the upper seed. × 12. E. Capsule lid as shed from the plant, showing the remains of the flower and the placental tissue still in position. × 12. F. Upper seed. Dry. × 33. G. Same seed after soaking in water for ten minutes. × 33. H. One of the lower seeds from the same capsule as the upper seed shown in Fig. 1 K. Face view. × 33. J. The same seed in edge view. × 33. K. Upper seed, edge view. × 33. L. The same seed in face view. × 33. M. Lower seed dry. × 33. N. Same seed after soaking in water for ten minutes. Compare the mucilaginous development of the testa of the seed with that of the upper seed shown in Fig. 1 G. × 33.

Seeds of both kinds were measured and the accompanying diagram (Fig. 2) of the variation in the length and breadth of both upper and lower seeds shows that there is very little overlap in the dimensions of the two kinds of seed; if the breadth of the two be compared it will be apparent that while the mode for the upper seeds is about 0.52 mm., that for the lower seeds is 0.72 mm., and the length of the two types of seed also shows a marked difference, for here the mode for the lower seeds is 1.16 mm., while that for the upper seeds is only 0.9 mm.

The external differences of shape and size of these two seeds is apparently not correlated with any difference in internal structure; both seeds show a typical dicotyledonous embryo surrounded by endosperm.

Striking as is the difference already referred to, in the shape and size of these two seeds, a difference which may be due entirely to their position in the capsule, the behaviour of the seeds in water is of even greater interest, and leaves little doubt that the seeds in this plant are both morphologically and biologically dimorphic.

When they are thrown into water they behave in a strikingly different manner. The large seeds from the lower part of the capsule absorb water very rapidly, with the result that after about 1 minute the seed is surrounded by a very conspicuous mucilage sheath. This mucilaginous layer continues to enlarge for some time, and in seeds soaking in water the mucilage of adjacent seeds will run together so that the whole forms a gelatinous mass. These seeds remain floating on the surface of the water only for a very short time, the majority sinking after the first minute or so, and none of them remains afloat for more than 35 minutes (Fig. I, M, N).

The seeds from the top of the fruit, on the other hand, take up water much more slowly and do not show anything like a comparable development of mucilage, even after prolonged immersion (Fig. I, F, G).

In addition to this difference these smaller seeds will remain floating for more than 48 hours, at the end of which time they have begun to germinate; after a similar period of immersion the large seeds have not germinated. This difference in the time of germination of the two types of seed in water is almost certainly associated with the fact that as the large seeds sink so rapidly there is a limited supply of oxygen available and thus germination is retarded, while the small seeds which remain on the surface have a sufficient supply of oxygen. This view is supported by the fact that when seeds are soaked in water and put on moist filter paper or damp sand, both will germinate with equal rapidity.

The difference in buoyancy of the two kinds of seed is apparently quite considerable, since they behave in a similar manner when a 3.5 per cent. solution of Tidman's sea-salt is substituted for fresh water. The large seeds sink quite quickly while the small seeds float for a very long time,

but as under these conditions they do not germinate they will remain afloat indefinitely.

The floating capacity of the two types of seed can almost certainly be correlated with a different degree of mucilaginous development of the testa when the seeds are in contact with water, for when large seeds are freed from mucilage they float and germinate on the surface with the same readiness as the small seeds.

In the genus *Plantago* as a whole the development of mucilage on the seeds is a common feature, and the striking difference in degree of development in these two types of seed in *P. Coronopus* led to an attempt to elucidate the possible function of the mucilage apart from its probable value as a means of dispersal.

Kerner ('Natural History of Plants', Vol. I, p. 615) points out that many seeds possess a mucilage which only appears when the seed is moistened, and that the primary use of this is to cement the seed to the soil where it will germinate.

Griffiths ('A Novel Seed Planter'—Torrey Bot. Club, 1902, p. 164) in describing the role of the mucilage in *P. fastigiata* states that 'the primary function of the mucilage is to bury the seed'. As will be shown later, the rapidity of germination and the effect of light on germination suggests that in *P. Coronopus* the primary role of the mucilage is not likely to be that of burying the seed.

Fifty seeds were soaked in tap water and subsequently freed from their mucilage by rubbing on dry filter paper. These were planted on moist sand in an earthenware dish and covered with a sheet of glass: the whole was kept in the open air in full illumination. Another fifty seeds were planted in a similar manner, but these had the mucilage untouched. Three days after planting the germination commenced in both sets, and 24 hours later a high percentage of seedlings had been produced. In all the young plants developed from the seeds with mucilage, the initial stage is the emergence of the radicle followed by a definite and well-marked arching of the hypocotyl. Subsequently, although the seed had been sown on the surface of the soil, the radicle penetrated it and the cotyledons and plumule were pulled out of the testa which remained on the surface of the sand.

In the seedlings grown from seeds without mucilage the initial stage as before is the emergence of the radicle, but the arching of the hypocotyl is not so definitely marked, and the radicle shows a greater tendency to remain on the surface, the first ring of root hairs being clearly visible. The cotyledons and plumule, as in the other seeds, are drawn out of the seed coat; thus the mucilage appears to function in holding the seed in position in the soil, thereby enabling the radicle to force its way between the particles.

Direct proof of the efficiency of the mucilage as a dispersal mechanism

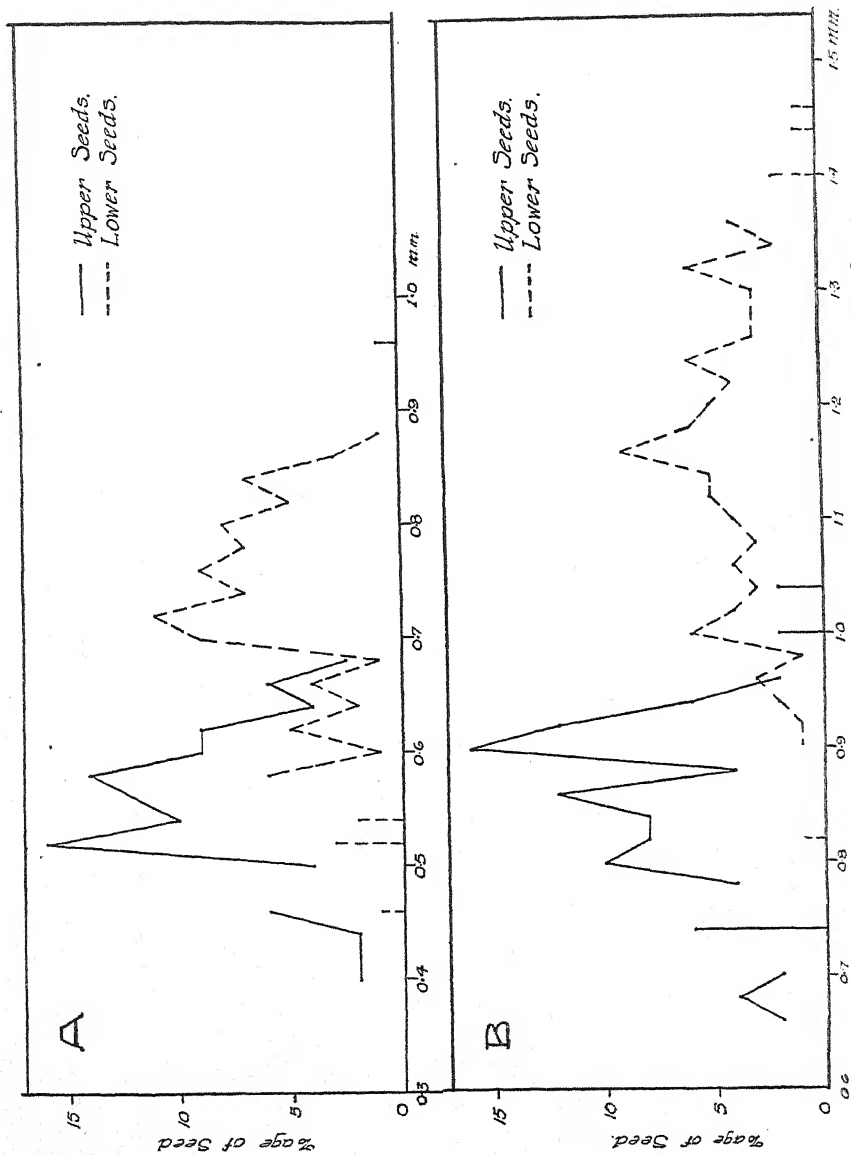


FIG. 2. Variation in length (A) and breadth (B) of upper and lower seeds.

is difficult to obtain, but it doubtless does play some part in aiding the distribution of the seeds. This species, however, seems to have another efficient mechanism for the dispersal of its small seeds. As has already been stated, these seeds remain within the capsule lids when these fall from the plant. The lids with the contained seeds are as buoyant as the small seeds themselves, consequently there is every reason to suppose that the whole lid may be distributed by water as readily as the seeds and in addition, since the whole is relatively light and not sticky, it may quite readily be blown about by the wind. Quite apart from this benefit to the plant there is another which is equally important, namely, the delay in germination which the retention of the seed affords.

In nature the plant sheds its large seeds in considerable numbers towards the end of the summer when they germinate immediately, and the only safeguard against destruction appeared to be the long flowering period which results in some seeds maturing after the main crop. It has been observed, however, that when there were a number of well-developed seedlings round the parent plants there were also a number of capsule lids containing the small seed. One hundred of these small seeds were planted on damp sand at the same time as a similar number of lids containing seeds. These were kept under observation, and after nine days 99 per cent. of the seeds had germinated. At the same time only two of the seeds from the capsule lids had produced seedlings, and at the end of three weeks there were only eight seedlings. Unfortunately, the experiment had subsequently to be abandoned, but it serves to show that the retention of the small seed in the capsule lid does materially delay its germination.

The large seeds of this plant in nature germinate as soon as they are shed, usually about September, but they will germinate equally well at all times of the year, and they retain their vitality for a considerable period. The following table shows the percentage germination for seed from one plant collected in August 1927. The first sowing of seed was in October of the same year when the larger seeds gave 100 per cent. germination after three days. Five months later the germination had fallen somewhat, but after more than three years a germination of 90 per cent. was still obtained. This figure, however, was only reached about three months after planting, while in the earlier trials 90 per cent. of the seeds had germinated in three weeks. At this same time after planting in the last trial more than 70 per cent. of the seeds had germinated.

Age of seed at sowing.	Description of seed.	Date of sowing.	Date of final germination.	Percentage germination.
2 months after ripening	Large seed.	14. 10. 27	17. 10. 27	100
" " "	Small seed.	14. 10. 27	17. 10. 27	93
5 " " "	Large seed.	16. 1. 28	6. 2. 28	90
" " "	Small seed.	16. 1. 28	6. 2. 28	93
45 " " "	Large seed.	29. 4. 31	13. 7. 31	90

The difference in the time taken to attain the maximum germination may perhaps be explained by the different time of year at which the seeds in the above experiment were planted. Seeds planted in June reached 100 per cent. germination after 43 days, while other samples collected at the same time and planted in September reached their maximum germination in 13 days, and more than 95 per cent. had germinated in 5 days.

From the above experiments, therefore, it is apparent that the seeds of this plant can germinate at all times of the year, although they do so most rapidly in September and October. Further, the seeds retain their power of germination for a very considerable period.

A comparison of the accompanying figures for the germination of seeds with and without mucilage at different seasons of the year in both light and darkness shows that in September, which is the time at which germination takes place most easily and most quickly, there is no appreciable difference in the rate of germination under varying conditions (Fig. 3). In May and June the presence of the mucilage of the seed coat apparently accelerates the germination of the seeds in the light while it retards their germination in the dark.

*Plantago Coronopus* is characteristically a plant of sandy soils and is frequently littoral, the form *pygmaea* being often associated with saline soil, so the effect of salt on germination and vitality was tested.

The seeds will not germinate in soils of high salinity watered with 4 per cent. Tidman's solution, but germination, though markedly delayed, will take place in soils watered with 2 per cent. Tidman's solution. On the other hand, a high salt content does not apparently injure the seeds which germinate when the salt concentration is suitably lowered.

In spite of the ease with which seeds germinate under normal conditions it was found difficult to raise plants owing to the damping off of the seedlings. When, however, surface watering was replaced by a capillary water-supply the difficulty was overcome, and healthy plants were readily grown. Whether this demand for a capillary water-supply has any bearing on the observed fact that *P. Coronopus* has apparently disappeared from many inland localities from which it had previously been recorded is uncertain, but it has been suggested that since the young plants grow better with a capillary water-supply the lowering of the permanent water table may have influenced the growth of *P. Coronopus* in inland habitats.

In addition to reproducing extensively by seed, the plants produce axillary offsets on the short erect underground stem. These frequently remain attached to the parent axis, but can, if separated from it, readily produce new plants.

Externally the upper part of the axis is covered with leaf bases which gradually decay leaving the stem with closely set leaf scars. The axis shows a number of constrictions between which it is considerably swollen.

Percentage germination of lower seeds (average of 400 seeds).

Day.	May.				June.				September.			
	Light.		Dark.		Light.		Dark.		Light.		Dark.	
	With mucilage.	Without mucilage.	With mucilage.	Without mucilage.	With mucilage.	Without mucilage.	With mucilage.	Without mucilage.	With mucilage.	Without mucilage.	With mucilage.	Without mucilage.
1	0	0	0	3	0	0	0	3	0	0	0	0
2	28	5	0	14	1	5	1	11	3	1	12	1
3	49	10	0	23	5	20	19	41	63	18	78	41
4	65	21	5	25	—	—	—	—	89	45	87	65
5	79	60	7	27	—	—	—	—	96	70	94	78
6	82	65	8	27	74	33	59	72	—	—	—	—
7	83	66	8	28	74	33	60	72	97	80	98	86
8	83	66	8	28	76	36	61	73	97	86	98	87
9	83	68	8	28	77	39	65	74	97	90	98	88
10	83	68	8	28	77	39	66	75	98	91	99	88
11	89	71	10	38	—	—	—	—	98	92	99	89
12	95	73	13	43	—	—	—	—	99	93	99	89
13	97	79	23	49	42	42	77	82	99	94	99	90
14	97	80	24	50*	43	43	80	84	—	—	—	—
15	97	80	25	50	43	43	83	84	—	—	—	—
20	97	83	27	57	51	51	89	91	—	—	—	—
25	—	—	—	—	86	86	95	96	—	—	—	—
30	—	—	—	—	—	—	—	—	—	—	—	—
34	98	87	48	81	—	—	—	—	—	—	—	—



These swellings are apparently the result of successive annual contractions

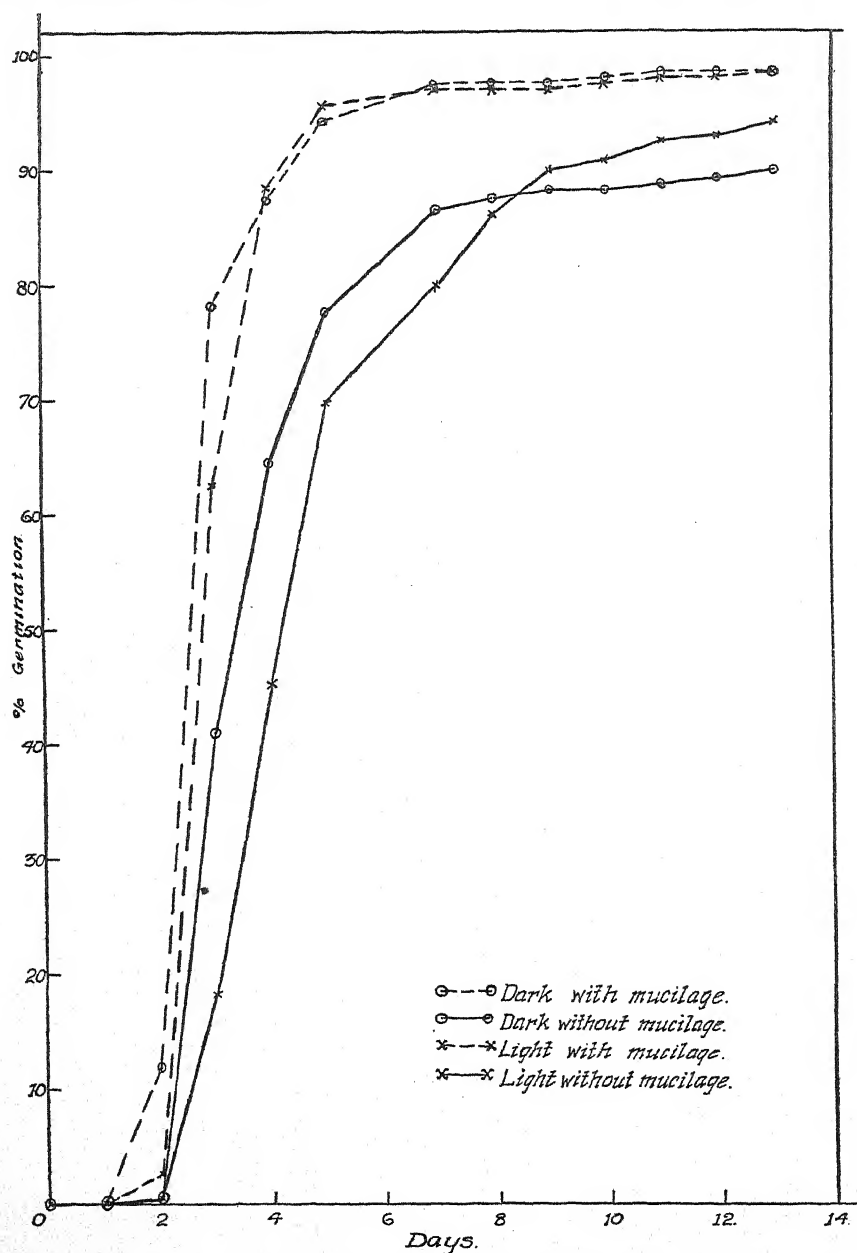


FIG. 3. Germination of lower seeds. September.

of the axis in bringing the rosette to soil level. The correctness of this assumption has been confirmed by examination of plants of known age

and by a study of their annual rings. By this means it was found that many plants were several years old.

#### SUMMARY.

The ovary of *P. Coronopus* is bi-carpellary and bi-locular, with a septum between the two loculi in a plane tangential to the axis. In a median transverse section four ovules (from which four seeds are normally produced) are usually seen, of which two are present in each loculus. In the anterior carpel, however, a third ovule is always present, situated above the two lower ovules and separated from them by an outgrowth of placental tissue similar to that which separates the lower seeds from one another.

An outstanding feature is that the upper and lower seeds differ both in shape and size, and measurements of a number of seeds show that there is very little overlap in either the length or breadth of these two types.

The upper and lower seeds behave very differently in water, the lower and larger seeds absorb water very quickly and within two minutes of contact with water develop a very conspicuous mucilage sheath; these lower seeds sink in water very rapidly. The upper seeds, on the other hand, remain floating for 48 hours, when they begin to germinate, and even after this prolonged immersion they do not produce a comparable amount of mucilage.

Although the seeds of this plant are both morphologically and biologically dimorphic, there is no difference in internal structure and no difference in their capacity to reproduce the species.

The large seeds, of which nearly all are viable, are shed in the autumn and germinate almost immediately. The small seed is shed, still enclosed within the capsule lid and held in it by the placental core. When these seeds are collected, they germinate as readily as the lower seeds, but in nature the retention of the seed in the capsule lid effectively delays its germination. It seems probable also that by its retention within the capsule lid, the small seed is provided with a dispersal mechanism which differs from that of the large mucilage-covered lower seeds.

Experiments to test the effect of the mucilage upon the germination of the seeds are reported. The results suggest that the effects vary with the season of the year.

The seeds will not germinate in highly saline soil, but they are apparently not injured by salt.

*P. Coronopus* produces axillary offsets capable of reproducing the plant vegetatively.

Many of the plants are perennial and show swellings on the axis which apparently result from successive annual contractions in bringing

the rosette to soil level. The number of these swellings corresponds with the number of rings in the wood of the root, and with the known age of the plant.

In conclusion, I would like to take this opportunity of expressing to Professor T. G. Hill and Professor E. J. Salisbury my thanks for their helpful criticism and advice during the course of this work.

# The Method of Germination of Seeds Enclosed in a Stony Endocarp.

BY

ARTHUR W. HILL, K.C.M.G., F.R.S.

With twelve Figures in the Text.

A PRELIMINARY account of the investigations relating to those fruits which have a stony endocarp was given before the Linnean Society on December 13, 1917. Since that date pressure of other work has prevented the completion of the account, but the delay has enabled some additional material to be obtained and examined, thanks to the kind assistance of Mrs. Clement Reid (*Nyssa* and *Mastixia*), Dr. I. B. Pole Evans, C.M.G. (*Sclerocarya*), The Chief Conservator of Forests, Madras (*Tectona*), and Mr. F. L. Squibbs, Assistant Director of Agriculture, Seychelles (*Northea seychellana*). Many of the drawings were made originally by myself, but all the finished drawings reproduced in this paper have been made by Mr. G. Atkinson, our botanical artist at Kew.

My thanks are also due to Mr. L. A. Boodle in connection with the determination of the fossil *Mastixia* seeds sent by Mrs. Clement Reid, and to Mr. Boodle and to Dr. Metcalfe for cutting sections of a few of the seeds to show the structure of the endocarps and their valves.

The seeds of plants bearing fleshy fruits or pseudo-fruits—tomato, mulberry, apple, &c.—are as a rule enclosed in some definite protective covering. This, in the case of the tomato or of gourds, for example, takes the form of a fairly strong bony envelope, which is the lignified outer seed coat or testa. In a large number of plants, however, the testa has remained thin and papery and the protective function has been assumed by the endocarp, the innermost layer of the fruit wall.

In such cases, of which the plum and cherry (*Prunus*), the coconut and other palms, are familiar examples, the endocarp has been developed into a hard bony structure, completely enclosing the delicate seed, and the seed thus lies in a lignified envelope or 'box', which appears to be so hermetically sealed that the method of emergence or escape of the contained embryo on germination becomes an interesting matter of observation and study.

Among the dicotyledons the different plants which exhibit stony endocarps may be grouped either according to the devices which have been evolved to permit of the escape of the embryo, or according to the number

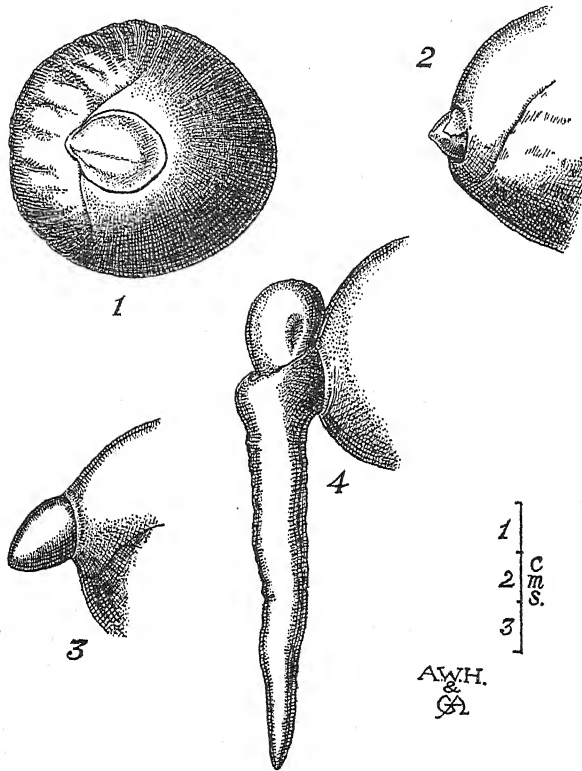


FIG. 1. *Northea seychellana* Hook. fil. 1. A fruit showing the cap-like covering of the orifice of the seed cavity. 2. The emerging radicle, the cap or valve having been pushed away. 3. A later stage. 4. The radicle and the elongated hypocotyls have carried the plumule out of the cavity and it is now emerging from between the stalks of the cotyledons.  $\times 2/3$ .

of loculi within the stony envelope, usually mis-called the 'seed'. The simplest type of liberation of the embryo is by the splitting into two halves of the stone or nut, as is seen in the peach, cherry, plum (*Prunus*) (10) or (Fig. 2) olive (*Olea*), which is derived from a single carpel, or by the walnut (*Juglans*), where the fruit is composed of two fused carpels (6).

Another device is exhibited by those stones which, instead of splitting, throw off a specially-prepared portion only of the hard endocarp, like a window shutter or fenestra, good single-seeded examples of which are afforded by *Nyssa* and by *Mastixia* (Cornaceae).

The large thick-shelled seeds of *Northea seychellana* (Fig. 1) show a small circular area at one end, which on germination is pushed off like a lid by the

emerging radicle of the embryo. Similar fenestral arrangements are shown by those species of *Cornus*, which have fairly large, bony 'seeds'. These are of interest, as they are examples of a two-seeded fruit with a stony endocarp (Fig. 5).

Several examples of three-seeded fruits exhibit fenestral openings,

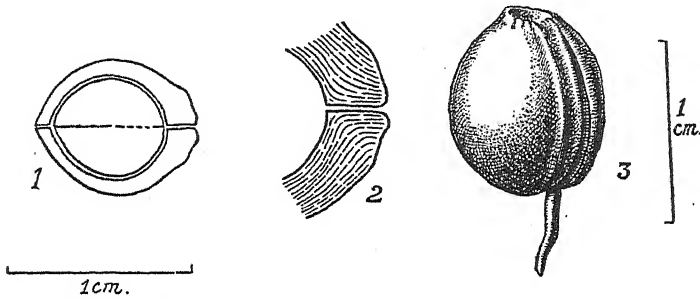


FIG. 2. *Prunus Cerasus* L. 1. The fruit in transverse section showing the line of cleavage of the stone. 2. Diagrammatic representation of the arrangement of the cells on either side of the cleavage line. 3. A germinating fruit showing the stone splitting into two halves.

such as the many species of *Canarium* (Burseraceae) and some species of *Elaeocarpus* (6). Teak, *Tectona grandis* L. f. (Verbenaceae) (11) bears a four-seeded fruit showing a somewhat similar contrivance for the liberation of the embryo, while *Saccoglottis* (Humiriaceae) and *Aubrya*, which closely resemble *Tectona* in their method of embryo-liberation, contain five seeds. *Davidia* (Cornaceae), with its many-seeded fruit, also throws off specially prepared portions of its stony endocarp in order to permit of the escape of the radicles of the germinating embryos (Fig. 12).

A third and more specialized method or device to permit of the egress of the embryo is shown by the genera *Sclerocarya* and *Dracontomelon* (Anacardiaceae). Here a definite, more or less circular, orifice is developed over the cavity in the endocarp containing each embryo, which is closely fitted with a bony plug or stopper, and which has to be ejected before the radicle can emerge (Figs. 9 and 10).

The most complicated structure met with is the many-seeded, complex fruit of *Pleiogynium*, also belonging to the family Anacardiaceae, where the mesocarp has become stony in addition to the endocarp. On germination the hard mesocarp undergoes no appreciable change, and the embryos are liberated by the upper part of the endocarpal covering of each seed being thrown off in the form of a small conical cap or lid (Fig. 11).

In the simplest cases, exhibited by the genus *Prunus* (Fig. 2), the endocarp consists of two similar halves, which are so closely united that they cannot be separated until the plane of weakness, due to the arrangement of the cells of the endocarp along the median line of the stone, becomes softened. In the stone of *Prunus* the cells of the endocarp are arranged more or less parallel to the surface of the stone, and in transverse section have an oblong

outline, but near the median line, which marks the margins of the two halves of the stone, the cells tend to be arranged at right angles to their ordinary direction, and at the suture itself the cells lie parallel to each other

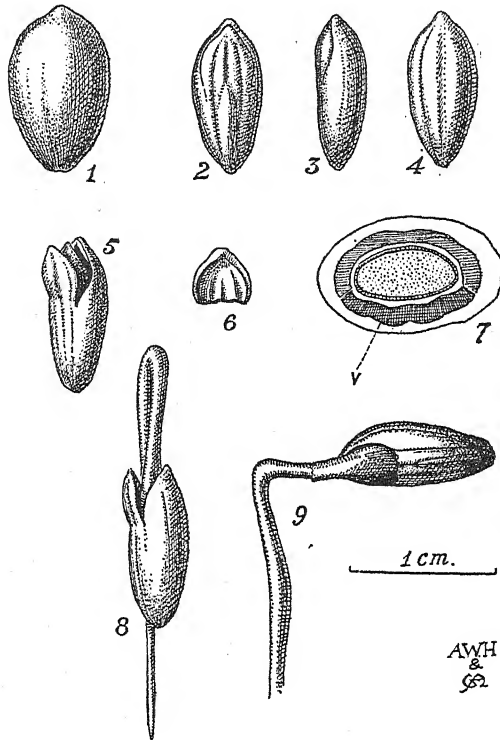


FIG. 3. *Nyssa sylvatica* Marsh. 1. The fruit. 2-5. The endocarp showing the valve or shutter in face or side view (2 and 3), and being pushed away by the emerging radicle (5). 4. Back view of the stone. 6. The valve detached. 7. The fruit in transverse section; the extent of the valve (*v.*) is shown by the hatching. 8 and 9. Seeds germinating with the valve pushed aside in 8 and completely thrown off in 9. All  $\times 3$  except 6 and 7, and to be compared with the fossil specimens of *N. sylvatica* of the Pliocene deposits of Swalmen and Reuver shown in Fig. 4.

in each half of the stone. The stone can thus easily be split into two halves after it has been well moistened as soon as germination commences, since there are no cells crossing the cleavage line which can tie one half of the stone to the other half (10).

When the stone is split in half the surface of the rim of the half-stone is seen to be smooth and unfractured.

The genus *Juglans* shows a similar arrangement, only here the stone or nut, which separates into two equal halves with smooth applied margins, is formed by the adhesion of two carpels, instead of being a single carpel splitting into two parts as in the case of *Prunus*. Here again the two

shells of the endocarp are not in definite organic connexion with each other, but each is a separate structure, the two component portions of the nut being closely applied together (6).

Other cases to be described are not of quite so simple a character, and the embryo can only be liberated from its stony protective envelope by the

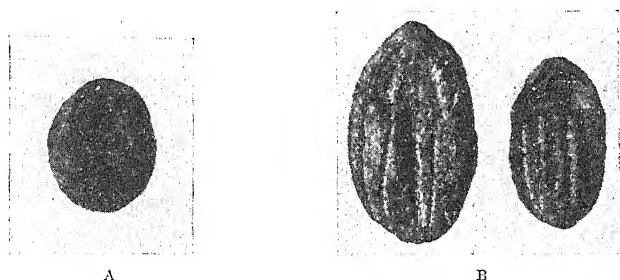


FIG. 4. *Nyssa sylvatica* Marsh. A. A fossil fruit from the Pliocene deposits, Reuver. B. Fossil specimens from the Pliocene deposits, Swalmen, showing the valves. From photographs kindly supplied by Mrs. Clement Reid.

detachment of a specially-prepared portion of the shell in the nature of a 'shutter' (fenestra) or valve. In such cases, which can be found in one-, two-, three-, four-, and many-seeded fruits, the portion of the endocarp which will be thrown off is marked out, as in the case of *Prunus*, in the early development of the fruit, and although the ripe dry stone shows no indication of where the valve or fenestra may be situated, a transverse section of the stone discloses the line of separation between the edge of the valve and the main portion of the stone. These valves in some genera tend to be broadly triangular-oblong in shape, with curved sides and a horizontal base (e.g. *Nyssa*, *Canarium*, &c.), while in others they are broadly fusiform and taper to a point at either end of the stone (*Tectona*, *Saccoglottis*, *Aubrya*, &c.).

*Nyssa* and *Mastixia* are good examples of one-seeded fruits which throw off a specially-prepared valve or fenestra, and the device appears to have escaped notice by former observers. They are also of interest since fossil specimens of the fruits of both these genera have been found by Mrs. Clement Reid in the lignite of Bovey Tracey, S. Devon, of probably upper Oligocene age, in the Hordle, Eocene beds, of the Isle of Wight and in the Pliocene beds of the Dutch-Prussian frontier (8). Fossil *Nyssa* seeds (*N. vestumni* Unger.) have been found at Bovey Tracey and at Swalmen and Reuver on the Continent, (Fig. 4) while *Mastixia* fruits with a single loculus, as well as a closely-related bilocular 'seed' with similar valve structures, possibly belonging to the Cornaceae, have been found both in the Hordle beds and at Bovey Tracey (7).

It is of interest to find that the most nearly related living species of *Mastixia*, judging from the seed structure, is *M. euonymoides* Prain, from



the Kachin Hills and Upper Burma, while *Nyssa* is now only known by some six species in the Eastern United States, the Himalaya and the Malayan Islands.

Fruits of *Nyssa* are figured and described by Gaertner (2) but no indication of the valve is given, and in fact he describes the putamen as

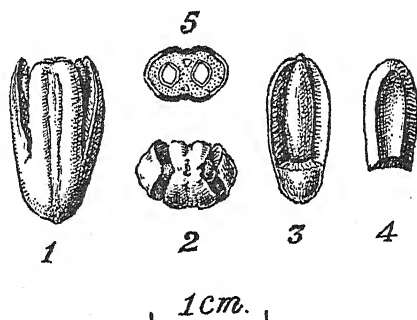


FIG. 5. *Cornus officinalis* Sieb. et Zucc. 1. The complete bilocular fruit showing the two valves commencing to split away. 2. A view of the apex showing the radicles of the two embryos pushing away the valves. 3. Side view with a valve completely removed exposing the seed. 4. The detached valve. 5. The fruit in transverse section with the two loculi. All  $\times 1\frac{1}{2}$ .

'evalve'; Wangerin (12) also figures the fruits of *Nyssa ogeche* and *N. sylvatica*, but omits any reference to the valve either in the text or the drawings. This perhaps is not surprising, as until the seed commences to germinate the plane of weakness between the valve and the rest of the endocarp is not apparent, the ribs being continuous down the whole length of the stone whether along the valve or not.

In *N. sylvatica* (Fig. 3) the valve is about one-third the length of the 'seed', broadly orbicular in outline with a bluntly triangular apex and a square-cut or slightly-rounded base. In the Pliocene fossils (see Fig. 4) the structure is identical and the valve has no doubt become visible owing to the softening and dissolving processes to which the stones were subjected when deposited in the estuarine mud. On germination, the cementing substance which firmly unites the valve to the rest of the endocarp dissolves and shows the outline of the valve very clearly, and as the radicle emerges the valve becomes completely detached and can be found lying loose in the soil.

The fossil *Mastixia* and also the 'seeds' of *M. enonymoides* show a very similar structure, and on cutting a transverse section through the upper part of the endocarp, where the valve occurs, the line along which cleavage will take place is clearly seen by the mode of arrangement of the cells at the edge of the valve, and in the adjacent tissue of the main portion of the endocarp from which it would separate on germination.<sup>1</sup>

<sup>1</sup> The unilocular fruits of *Commiphora allophylla* Sprague (Burseraceae) from Somaliland show a valve-like structure, somewhat similar to that of *Nyssa* and *Mastixia*, which probably becomes detached in the same manner on germination. See Hooker's *Icones*, tab. 3110, Figs. 3 and 4.

The 'seeds' of *Cornus officinalis* and of the bilocular fossil (? cornaceous) found by Mrs. Clement Reid in the Hordle beds resemble *Nyssa* and *Mastixia* in their mechanism, but the chief interest here lies in the fact that they contain two embryos, and a valve is provided for each loculus (Fig. 5).

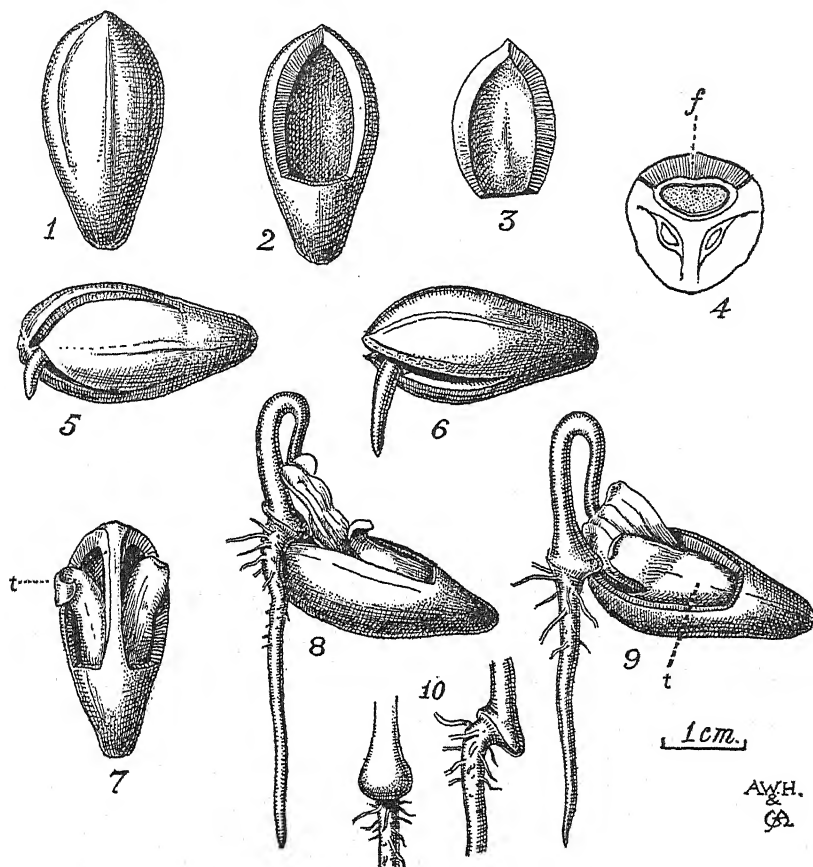


FIG. 6. *Canarium Schweinfurthii*, Engl. 1. The stone with one of the valves indicated. 2. The same with the valve removed. 3. The valve or fenestra seen from the inside. 4. The stone in cross section showing three loculi, only one fertile and the fenestra (*f.*) in section. 5 and 6. Two stages in germination, the fenestra being lifted up by the radicle. 7. A stone with two empty loculi and the remains of the testas (*t.*). 8. A seedling with its arched hypocotyl and the peg-like base of the cotyledons pressing against the edge of the stone. 9. A similar picture showing the cotyledons being drawn out of the testa and the peg of the hypocotyl pressed against the edge of the cavity. 10. The peg seen from the front and from the side. All  $\times 1$ .

The valve in *C. officinalis* is a stout structure, ovate-oblong in outline, with a truncated base and concave on the inner side where it wraps over the embryo.

*Canarium* (Burseraceae), with its three loculi, is somewhat similar to *Cornus* in that the hard, woody valves are triangular-ovate, pointed at the apex and horizontal at the base. On the inside they are concave with

a slight median ridge. The valve is about two-thirds the length of the 'seed', and the embryo cavity occupies the full length of the seed vessel, with the radicle lying at the broader upper end. On germination the

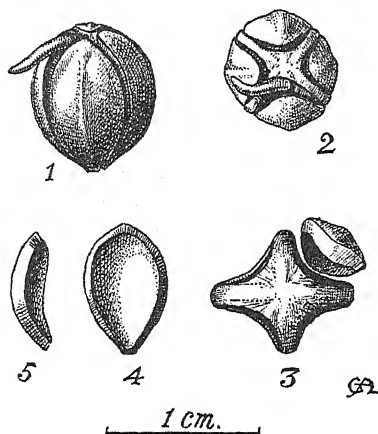


FIG. 7.

FIG. 7. *Tectona grandis* Linn. fil. 1. Fruit with outer coverings removed showing the four valves and the emergent radicle of one seedling. 2. The same from above to show the four valves and the ribs of the stony endocarp. 3. The skeletal core of the stone and one carved valve detached. All  $\times 2$ .

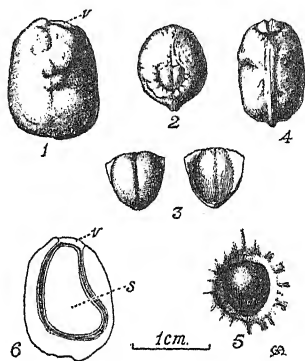


FIG. 8.

FIG. 8. *Haematostaphis Barteri* Hook, fil. 1. The stone after the removal of the fleshy pericarp showing the 'lid' (v.) or valve at the apex. 2. Apical view, showing the valve with its median groove. 3. The valve seen from the outside and from the inside. 4. The commencement of germination. The valve splits into two halves which are pushed aside by the growing radicle. 5. The valve removed showing the apex of the radicle lying in the cavity of the stony endocarp. 6. The stone in section showing the valve (v.) and the seed (s.) in the cavity.

growing embryo pushes the flange or valve slightly outwards and, in order to obtain sufficient leverage for the extraction of the cotyledons, a peg-like structure is developed at the base of the hypocotyl which rests on the top of the stone at its apex and the arch of the hypocotyl is thus able to force up the valve and draw out the cotyledons from the cavity. Owing to the radicle being well fixed in the ground this peg or foot may be said to stand on the stone and keep it pressed on the soil so that the arch of the hypocotyl can exert its full pressure for extracting the cotyledons; otherwise the stone might be raised into the air with the cotyledons firmly imprisoned within the endocarp (1).

Teak (*Tectona grandis*—Verbenaceae) is of interest, since the stony endocarp encloses four loculi, although as a rule only two or three of the ovules are fully developed (Fig. 7). The mechanism resembles that of *Canarium* and also of *Saccoglottis* and *Aubrya*; the small ovoid valves which split off are similar in shape to those of the two latter genera, and extend nearly the whole length of the endocarp (11). When the valves have split off, on the germination of the embryos, only the central core and ribs of the stony endocarp are left—much as in *Davidia*, to be described later.

The devices exhibited by the five-locular fruits of *Saccoglottis* and *Aubrya* (Humiriaceae) are of a similar character, except that in both the valves are of a more spongy nature and ovate in outline, rounded at one

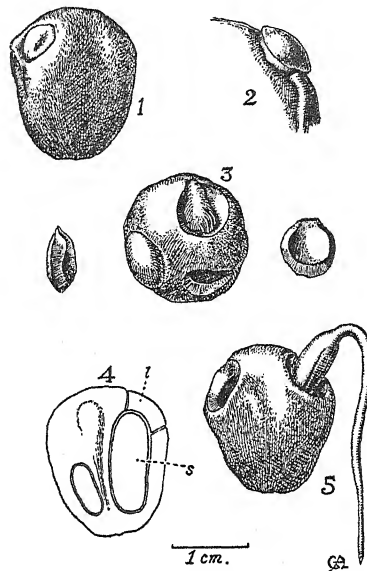


FIG. 9. *Sclerocarya caffra* Sond. 1. The stone after removal of the fleshy outer coats of the fruit. Two of the three apical valves or lids are shown. 2. The same with one of the lids being lifted by the germinating seed. 3. Stone seen from above with two lids removed to show the position of the germinating embryo—on either side or lids one side view to show its thin portion over the radicle, the other to show the concave inner surface. 4. The stone in section showing the relation of the lid (*l.*) to the rest of the endocarp; *s.* seed. 5. A young seedling, the cotyledons being drawn out of the cavity.

end and somewhat pointed at the other, deeply hollowed out on the insides to allow sufficient space for the embryos lying within close to the centre of the fruit.

Turning to the cases where the cavity or cavities in the endocarp are closed or plugged by a close-fitting stopper, the fruit of *Hippuris*, with its unilocular seed, may be cited as the simplest case. Here, as R. D'O. Good describes and figures (3), the endocarp has the form of an ovoid bottle, corked at the neck by a plug or stopper of hardened tissue. On germination, the stopper, which completely shuts in and protects the embryo within the cavity, is forced out by the developing radicle.

The family Anacardiaceae presents some of the most remarkable examples of seed protection by means of a hard woody endocarp, as well as some of the most ingenious devices to allow of the escape of the germinating embryos. In the genus *Haematostaphis*, with its single species *H. Barteri* indigenous in Nigeria, a small lid-like structure is seen at the apex of the 'stone' flush with the general surface and only visible after careful removal of the flesh of the fruit (Fig. 8). The unilocular endocarp is

covered by a felt of short stiff erect hairs. The lid, which is ovoid in outline and is marked by a slight median line or shallow groove, forms a close-fitting stopper, hermetically sealing the orifice of the cavity in the endocarp, in which lies the embryo with its radicle directly under the lid.

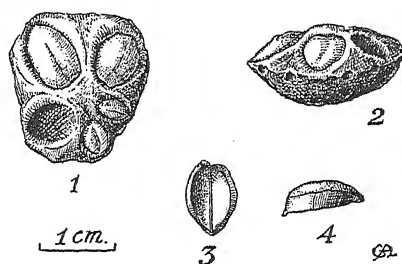


FIG. 10. *Dracontomelon mangiferum* Blume. 1. Surface view of the stone after removal of the fleshy pericarp and mesocarp showing the lids closing the cavities in the stone which contain the ovules; one lid has been detached. 2. The stone from the side with two lids in position. 3. A lid detached seen from the inside. The concave inner surface is polished and marked by a median ridge. 4. The lid from the side, the thin edge is nearest the outer edge of the stone. All  $\times 1\frac{1}{2}$ .

On germination the lid separates into two equal halves which are pushed apart and thrown aside by the radicle as it emerges. When dry it is impossible to remove the lid, and it is only after treatment with acetic acid that it can be dislodged, nor is there any clear indication of the double nature of the lid before the seed germinates (1).

The genus *Sclerocarya* (Anacardiaceae), of which the Kaffir plum, *S. caffra* Sond., affords a good example, is somewhat similar to that of *Haematostaphis*, though here there are three loculi, each with its lid or stopper (Fig. 9). The large, woody, roughly-suborbicular stone, contained within the fleshy fruit, is about 2 cm. in diameter, and the outer walls of the three loculi are 3 mm. or more in thickness. Each orifice is hermetically sealed by a woody plug or lid 3 mm. thick, curved on the outer side, and hollowed out and polished on the inner side. The plug or cap-like lid is thinned out at the lower corner, and from the inside looks to be provided with a small beak-like spout. This marks the spot where the developing embryo will push up the cap with the pressure of the growing radicle, and is no doubt the weak spot in the armature, through which external moisture enters when the 'seeds' are placed in a condition favourable for germination.

*Dracontomelon* (Anacardiaceae), the 'seed' which started this investigation,<sup>1</sup> exhibits a mechanism similar to that of *Sclerocarya* (Fig. 10). The

<sup>1</sup> It is of some interest to record the commencement of the present investigation. During the war (1917) some fruits were picked up in Limehouse, East London, after an air raid, and were sent by the Home Office to the Pharmaceutical Society for investigation and by the Society to Kew for determination. Since they were succulent and edible, it was thought they might be poisonous and

stony endocarp differs principally in being more compressed or flattened and is pentagonal to hexagonal in outline; it encloses five loculi, sealed by lids, like those of *Sclerocarya*, on the upper surface of the 'stone', which are

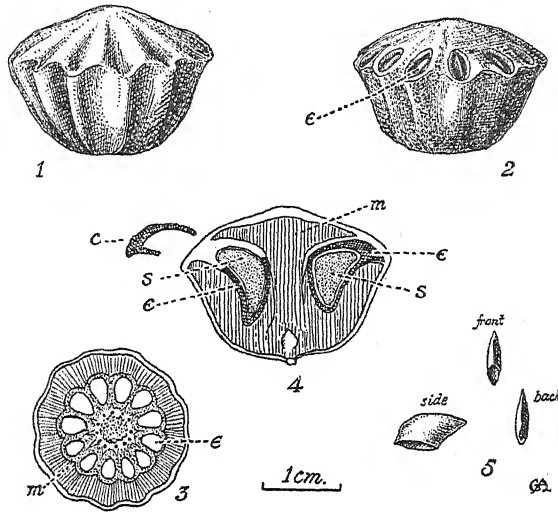


FIG. 11. *Pleio gynium Solandri* Engl. 1. The stone with outer covering removed, a fluted basin-like structure with a domed upper surface. 2. The same to show the orifices in the stony mesocarp with the apices of the endocarp caps (e.) within the cavities. 3. The fruit in transverse section (m.) stony mesocarp, (e.) endocarp with cavities containing the seeds. 4. The same in longitudinal section (m.) mesocarp, (e.) endocarp with cap, (c.) detached and the seed (s.) lying in the cavity. 5. The cap or lid of the endocarp from the side, front and back. All  $\times 1$ .

relatively larger to the 'stone' than those in the allied genus. Each lid is ovate in outline with the subacute apex towards the edge of the stone. The full-sized lids are about 6.5–9 mm. long, 4–6 mm. broad, and about 1.5 mm. thick, the inner surface being concave. As in *Sclerocarya*, the lids are lifted by the radicles as they push upwards on the germination of the embryos, the cementing of the lids to the sides of the orifices being softened by the moisture imbibed by the 'seed' when sown in the ground (1).

*Pleio gynium* (Anacardiaceae) is of interest since the seeds, which are furnished with stony endocarps, are further protected by being enclosed in a strong stony mesocarp (Fig. 11). The whole stony structure, which encloses some twelve 'seeds', is somewhat turbinate in form and convex on the top, and measures about 2.8 cm. in diameter by 1.8 cm. high, the orifices in which

had been dropped with sinister purpose from an enemy aeroplane. They proved to be fruits of *Dracontomelon sinense*, and had possibly been dropped by some sailor home from the East.

It was the examination of these fruits and the investigation of the remarkable structure of the endocarp with its five cavities sealed by plugs or lids that led to a more comprehensive survey of similar contrivances being undertaken from time to time.

the seeds are embedded being arranged round the margin on the upper surface. The endocarp is divided into a lower tubular portion, which is more or less fused with the tissues of the mesocarp, and an upper cap, which is firmly cemented to the lower portion but becomes detached and is thrown off on germination. The dome-like upper surface of the stony mesocarp arches over the tops of the endocarps to some extent and affords a strong extra protection, leaving only quite small orifices through which the endocarpic caps can be pushed. Each lid or cap is similar to those of *Sclerocarya*, but is triangular in section with a sharp ridge on the upper surface. The cap within is hollowed out to contain the upper part of the embryo and is pointed in front, that is, in the direction of the outer side of the orifice of the mesocarp. Whereas the cap of *Sclerocarya* might be compared in shape to a circular military cap, that of *Pleiogynium* somewhat resembles a French forage cap. The cap measures at the base about 6 mm. long by 1.5 mm. broad, and is about 4.5 mm. in height (10).

*Davidia* (Cornaceae) is another example of an interesting type of stony endocarp enclosing several seeds (Fig. 12).

In *Davidia*, as in *Pleiogynium*, there is no possibility for the seedlings to get away from the stone on germination, and although in both cases several seedlings may result from each stone, only one is likely to survive in the struggle for existence in the course of a few weeks after germination. These two plants, therefore, despite the fact that they have evolved a most efficient method of seed-protection and have developed ingenious devices for the liberation of the embryos, have apparently somewhat defeated their object, since so many good seedlings must perish when the fruits of either *Davidia* or *Pleiogynium* germinate.

An extreme case of this wastage of effort owing to the intensive development of a protective outer covering is, of course, furnished by the cannon-ball-like fruit of the Brazil nut (*Bertholletia*), where only one seedling ultimately survives from among the numerous (15-20) potential seeds or nuts, enclosed in the hard, woody endocarp, which struggle to germinate (13). *Lecythis* would probably furnish a similar story.

The germination of the fruits of *Davidia* was described and figured by the late Dr. W. B. Hemsley<sup>1</sup> soon after they were sent over to England, and as both he (4) and Horne (5) describe the manner of germination fully there is not much to be added to their accounts. The endocarp consists of a hard, bony, central axis with radiating flanges, and in the triangular spaces or grooves between these flanges or ribs lie the embryos. The embryos are securely walled in by hard endocarp tissue which bridges across the spaces between the extremities of the ribs, and in the lower

<sup>1</sup> Since Hemsley's figures (4), especially Figs. 2, 5, and 7, do not show the structure very clearly, I have added some fresh figures depicting the details of the structure of the *Davidia* endocarp and its valves.

portion of the endocarp the tissue is continuous with that of the general skeletal structure of the endocarp. When dry, the whole endocarp appears to be a solid structure, but when germination is about to commence it is

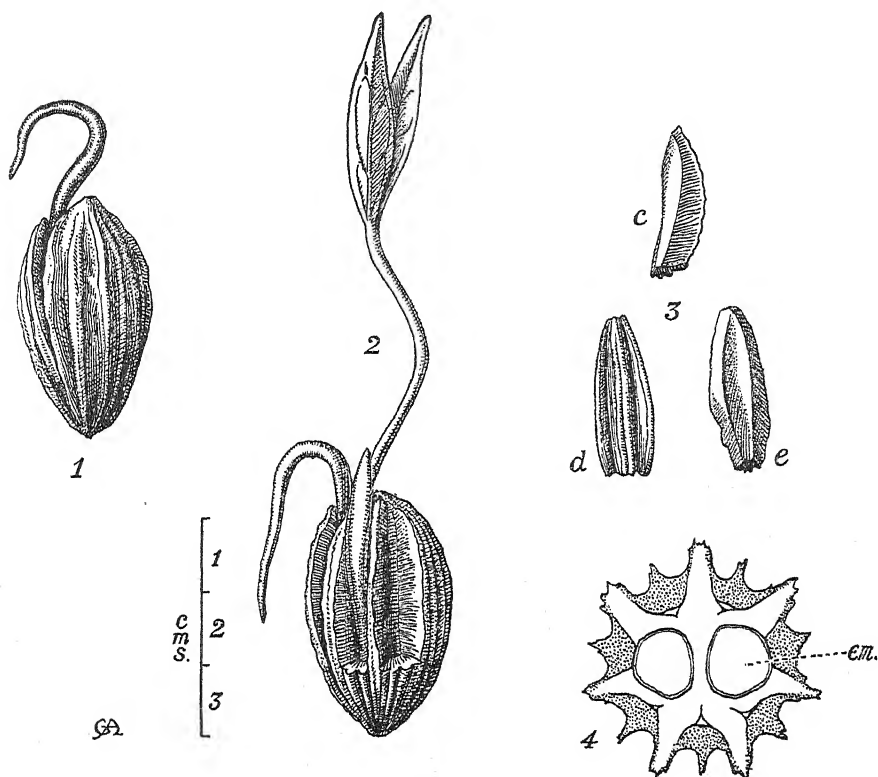


FIG. 12. *Davidia involucreta* Baill. 1. The stone with one germinating seed. The radicle is pushing aside one of the grooved valves or fenestrae of the endocarp. 2. A later stage in germination showing three seedlings and the cavities in which the embryos lie. 3. The valves, (c.) from the side, (d.) the exterior appearance with grooves continuous with the grooves on the lower part of the stone, (e.) from the inside showing the concave inner surface, the pointed apex and the truncated base. 4. A stone in transverse section showing seven loculi, only two in this case fertile. The valves thrown off are indicated by the shaded portion, (em.) embryos.

seen that the upper portions of the bony bridging tissue between the ribs become loosened and can be detached, and are narrowly-lanceolate, shutter-like bodies, flat at the base and with a pointed apex.

The surface markings, ridges and grooves, of each shutter are continuous with those of the lower portion of the stone or nut, and until germination commences no trace of the horizontal line of separation at the base of the valve or shutter can be seen.

On germination, in addition to the shutter between the ribs being thrown off, a small portion of tissue from the outside of the ribs is also thrown off, and it would seem that this outer layer of the endocarp, which



extends over both ribs and valves, serves to cement the ovules all the more firmly and hermetically in their narrow cells.

The series of seeds enclosed in stony endocarps which have been discussed afford interesting examples of the care taken to protect the embryo until germination may become possible.

In the simpler cases of unilocular or bilocular fruits the protective methods evolved appear adequate and fully efficient, but in the more complicated cases, such as *Pleiogynium*, *Davidia*, and *Bertholletia*, nature appears to have overrun the mark and largely defeated the object in view, since only one out of many possible seedlings can ever hope to survive. These reflections bring some other somewhat analogous cases to mind, as for instance, those Pines (*Pinus muricata* in particular), whose seeds do not normally escape from the cones until they have been subjected to the intense heat of a forest fire, or the Wattles whose seeds usually will not germinate until a fire has passed over the ground in or on which they lie;<sup>1</sup> though they will germinate after they have lain many years in the ground and the outer coat has partially rotted. Then, again, there are those seeds which cannot be induced to germinate unless they have been filed or scraped or treated with strong sulphuric acid, and when sown naturally may not germinate for many years until, like the Wattles, by some process of decay the hard protective testa has been modified and softened.

The whole subject of the seed, its power of lying dormant and the presence or absence of strong protective coverings, affords interesting material for careful study; why, for instance, should some seeds, apparently but slightly protected, such as Charlock (*Brassica Sinapis*), be able to remain viable for many years when preserved under suitable conditions?

Of quite another character are the fruits or seeds of the Mangrove, of *Crimum* and of *Typhonodorum*, for in these genera the seed continues its development without any resting period, and germination follows as part of a continuous process from the formation of the embryo. A somewhat similar case is afforded by the seeds of the Willows and Poplars, for they only retain their power of germination for a day or two after the seeds have become ripe, and it would seem that scarcely any resting stage can be tolerated in the almost continuous development of the embryo. Yet there are many other seeds, often with very delicate testas or protective fruit walls, which can retain their power of germination for relatively long periods, of which some of the Orchids present striking examples.

From the general appearance of a seed or fruit, it is hardly possible to predict whether it may remain in a viable condition for any length of time or not, nor can it be said that the seeds with stony endocarps are on the

<sup>1</sup> Tests made with the seed of *Acacia Baileyana* indicated that intense heat is required to soften the hard outer seed coat, which explains why large numbers of young Wattle plants appear after a bush fire. See Agric. Gazette N.S. Wales, xliii. Pt. 12 (1932), 947.

whole better adapted to retain their vitality than seeds which do not appear to be provided with any special protective coverings.

The various methods of seed protection, the nature of the life in a seed lying dormant over many years, and the reasons for immediate or long-delayed germination, afford problems all the more fascinating on account of their illusive character and because of the difficulties which attend our attempts towards their solution.

## SUMMARY.

An account is given of the structure of the stony endocarps of a series of fruits containing one to many loculi and of their mode of germination. They include *Prunus*, *Nyssa*, *Mastixia*, *Haematostaphis*, and *Northea*—one-seeded; *Cornus* with two seeds; *Canarium* with three, and *Tectona* with four seeds; *Saccoglottis*, *Aubrya*, and *Dracontomelon*, five-seeded and *Davidia*, *Pleiogynium*, and *Bertholletia* with several seeds. In *Pleiogynium* the mesocarp is stony in addition to the endocarp.

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# The Mechanism of Water Conduction in the Musci considered in Relation to Habitat.

## III. Mosses Growing in Dry Environments.

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With twenty-three Figures in the Text.

IN the first part of this investigation (3) the writer was concerned with an examination of the methods of absorption and conduction of water on the part of Musci which are normally found in wet habitats, while the second part (4) was devoted to similar work on Musci inhabiting situations which can be described as moist. The present, third instalment, is concerned with forms which are found in dry environments. Eight species have been investigated: *Hypnum cupressiforme* var. *filiforme*; *Dicranum scoparium*; *Hylocomium triquetrum*; *Ditrichum flexicauli* var. *densum*; *Anomodon viticulosus*; *Polytrichum commune*; *Mnium horum* and *Mnium undulatum*—and the sequence in which they are reported in the following pages corresponds to the order of the increasing drought of their habitats.

### I. *Hypnum cupressiforme* var. *filiforme*.

This variety of a species already examined (4) grows on extremely dry substrata, most commonly on the branches of trees. It is a very slender form, yellowish-green in colour, the individual plants being matted together and prostrate. The stems are long, slightly branched, the branches being straight, parallel and slender. Both the stems and branches are densely covered with small, regular, imbricating leaves which are falcato-secund or hooked (Fig. 1).

Experimental work on this and all succeeding mosses was carried out in a manner exactly similar to that described in Part I (3).

#### *Rate of external conduction.*

An investigation of the rate of external conduction in this form gave the following typical results:

TABLE I.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					24 hrs.
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	
1. (G. violet)	2.19	1.09	1.32	1.35	1.38	1.43	Tip
2. "	2.76	1.12	1.16	1.21	1.33	1.39	Tip
3. (Iron)	2.41	2.11	2.18	2.28	Tip	—	—
4. "	2.18	1.74	Tip	—	—	—	—
5. (Acid)	3.33	2.36	2.54	2.86	3.19	Tip	—
6. "	2.87	Tip	—	—	—	—	—

TABLE II.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	4.5	5 mins.	3.0
2.	5.6	15 mins.	4.2
3.	3.5	30 mins.	Tip
4.	4.5	1 hr.	Tip
5.	4.2	2 hrs.	Tip

The rate of external conduction is obviously very rapid, for the tables show that in some cases liquids conducted externally reached the tips of the plants in two hours and in some cases in thirty minutes. The rapid rate is apparently the result of the dense imbricating arrangement of the leaves which enclose narrow capillary channels between their adaxial surfaces and the stem.

*Amount of external conduction.*

Data obtained in investigating the amount of liquid conducted externally are given in Table III.

TABLE III.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	2.6	1 day	0.7
2.	2.4	"	0.22
3.	2.1	5 days	2.7
4.	1.7	"	1.99

The efficiency of external conduction is probably more apparent in the above table than in Tables I and II, for it is evident that 2.7 c.c. of water which had risen over a plant 2.1 cm. long in five days is a very considerable amount, which is obviously quite sufficient to meet all the demands of the plant.

*Rate of internal conduction.*

The internal conducting capacity of this moss is very small as a glance at Table IV will reveal.

TABLE IV.

Plant number.	Time.	Readings for potassium nitrate in cm.			Length of plant.	Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .		Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .	
1.	18 hrs.	3.5	0.7	0.9	4.2	0.5	1.0	
2.	1 day	3.0	0.5	1.1	5.1	0.5	1.2	
3.	3 days	4.5	0.6	1.2	4.2	0.6	2.0	
4.	"	2.2	0.4	1.0	3.9	0.2	1.9	

It is evident that with so effective an external conducting system the inability to conduct water internally affords little disadvantage to the plant. Moreover, the water available for epiphytic mosses such as the one examined is largely confined to their external surface, the rain-water accumulating in the dense moss tufts situated on the bark of the branches of the tree.

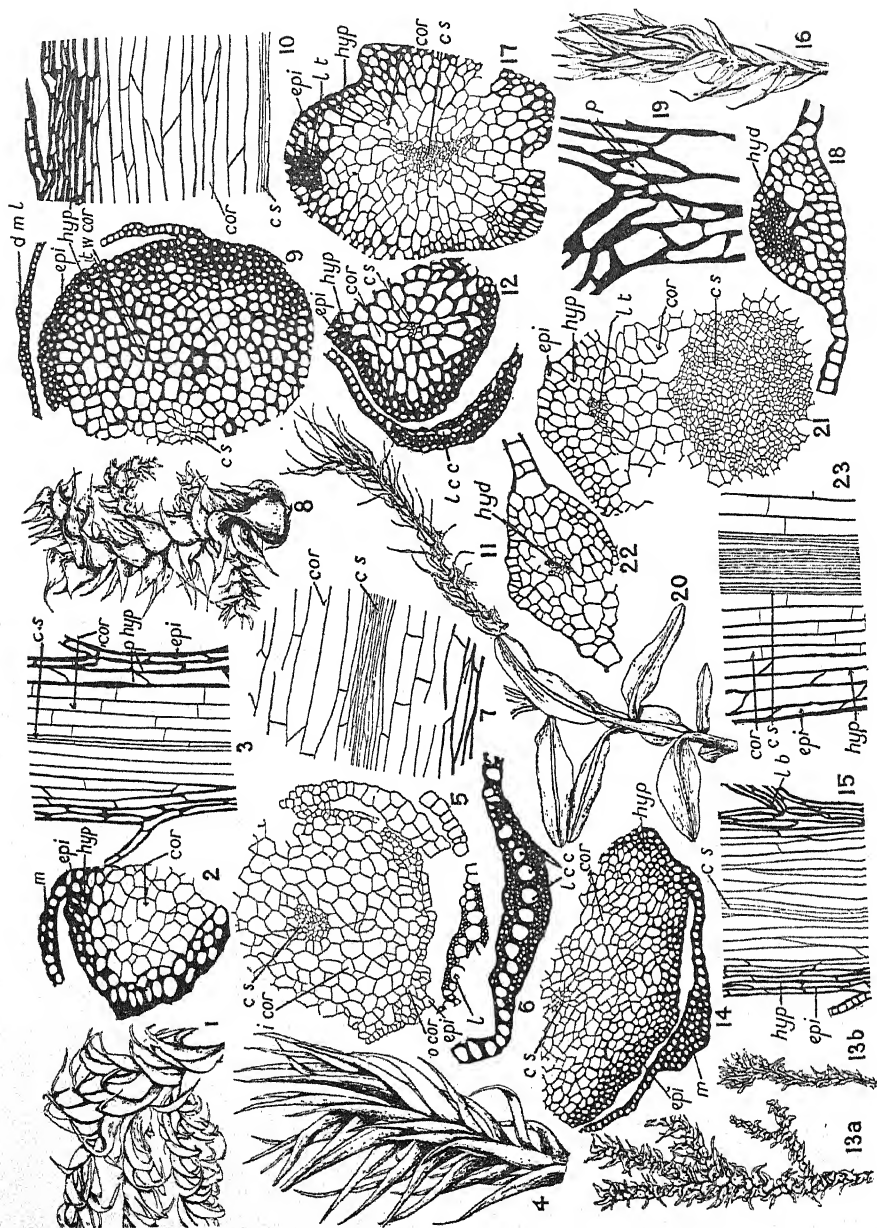
*Internal anatomy of the stem and leaf of Hypnum cupressiforme var. filiforme.*

Serial sections of the stem of this moss showed (Figs. 2 and 3) a thin-walled epidermis which becomes thickened and densely pitted in older stems; a thicker-walled pitted hypodermis; a large-celled, thin-walled cortex; and an occasional central strand of a few small, thin-walled cells. The central strand, when present, occurs in the main stem but is absent from the branches and consists of long, narrow cells with few transverse walls.

The leaf of this moss is small (Fig. 2) and consists of a single-layered lamina. The midrib is rarely present, but where it does occur it is double and confined to the basal part of the leaf-blade.

*Entry of materials and path of internal conduction.*

Entry of materials into the stem of this moss occurred chiefly and most rapidly through the thin-walled epidermis and hypodermis at the tip of the plant, complete penetration of all tissues in this region being evident after the base of the plant had been immersed in a solution of potassium nitrate for one hour only. Penetration through the older, thickened regions of the stem was slower and the presence of the salts was only demonstrated after eighteen and twenty-four hours. It seems, therefore, that this method of conduction, whilst supplying the physiologically active parts of the



FIGS. 1-23.

plants with a plentiful supply of water, also prevents their loss of water and so lessens the need for an appreciable transport of water throughout the internal tissues.

## II. *Dicranum scoparium*.

This very characteristic moss grows very abundantly in the more exposed regions of heaths, occurring in almost pure associations and forming dense tufts often from twenty to forty centimetres high. The plants are long and firm, bearing numerous leaves which are closely compacted at some points on the stem, the intervening regions being bare, so that the plants have a tufted appearance. The basal two-thirds of the stem is intertwined with other stems, but the upper third is free and erect and crowded with numerous large leaves which are all oriented in the same direction (Fig. 4).

### *Rate of external conduction.*

Experiments showed that the rate of external conduction in this moss is exceptionally rapid as is shown by Tables V and VI.

The compact arrangement of the leaves with their slightly sheathing bases ensures the enclosure of channels between their surface and that of the stem. Moreover, the compact arrangement of the various plants assists in the formation and retention of these channels of conduction so that the whole mass acts as a reservoir for rain-water. The result of this

*Abbreviations for Figures:* *cor.*, cortex; *o.cor.*, outer cortex; *i.cor.*, inner cortex; *t.w.c.*, thick-walled cortex; *c.s.*, central strand; *epi.*, epidermis; *hyp.*, hypodermis; *p.hyp.*, pitted hypodermis; *hyd.*, hydroids; *l.*, leaf; *l.t.*, leaf-trace; *m.*, midrib; *l.c.c.*, large central cells of midrib; *d.m.l.*, double midrib of leaf; *p.*, pits.

FIGS. 1-23. 1. Portion of the gametophyte of *Hypnum cupressiforme* var. *filiforme* showing the arrangement and divergence of the leaves.  $\times 55$ . 2. Transverse section of a branch of *H. cupressiforme* var. *filiforme* showing the absence of small-celled central tissue.  $\times 240$ . 3. Longitudinal section of the stem of *H. cupressiforme* var. *filiforme*.  $\times 182$ . 4. Portion of the gametophyte of *Dicranum scoparium* showing the arrangement and divergence of the leaves.  $\times 45$ . 5. Transverse section of a young stem of *D. scoparium*.  $\times 100$ . 6. Transverse section of the leaf of *D. scoparium*.  $\times 137$ . 7. Longitudinal section of the stem of *D. scoparium*.  $\times 126$ . 8. Portion of the gametophyte of *Hylocomium triquetrum* showing the arrangement and divergence of the leaves.  $\times 2$ . 9. Transverse section of the stem and leaf of *H. triquetrum*.  $\times 77$ . 10. Longitudinal section of the stem of *H. triquetrum*.  $\times 80$ . 11. Portion of the gametophyte of *Ditrichum flexicauli* var. *densum* showing the arrangement and divergence of the leaves.  $\times 2$ . 12. Transverse section of the stem and leaf of *Ditrichum flexicauli* var. *densum*.  $\times 154$ . 13a. Portion of the gametophyte of *Anomodon viticulosus* showing the normal arrangement and divergence of the leaves.  $\times 2$ . 13b. Portion of the gametophyte of *Anomodon viticulosus* showing the arrangement and divergence of the leaves in wilted forms.  $\times 2$ . 14. Transverse section of the stem and leaf of *A. viticulosus*.  $\times 124$ . 15. Longitudinal section of the stem of *A. viticulosus*.  $\times 86$ . 16. Portion of the gametophyte of *Mnium hornum* showing the arrangement and divergence of the leaves.  $\times 23$ . 17. Transverse section of the stem of *M. hornum*.  $\times 68$ . 18. Transverse section of the leaf of *M. hornum*.  $\times 103$ . 19. Longitudinal section of the peripheral tissues of the stem of *M. hornum* showing the pitted walls of the epidermis and hypodermis.  $\times 223$ . 20. Portion of the gametophyte of *Mnium undulatum* showing the arrangement and divergence of the leaves.  $\times 25$ . 21. Transverse section of the stem of *M. undulatum*.  $\times 78$ . 22. Transverse section of the leaf of *M. undulatum*.  $\times 118$ . 23. Longitudinal section of the stem of *M. undulatum*.  $\times 84$ .



arrangement is that *D. scoparium* conducts water to the tip of its stem often within half an hour of the insertion of its basal regions in solutions.

TABLE V.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	4.58	Tip	—	—	—	—	—
2. "	3.54	2.34	2.5	2.6	Tip	—	—
3. (Iron)	5.09	3.95	Tip	—	—	—	—
4. "	3.54	Tip	—	—	—	—	—
5. (Acid)	4.53	3.55	3.6	3.78	Tip	—	—
6. "	3.12	Tip	—	—	—	—	—

TABLE VI.

Plant number.	Length of plant in cm.	Time.	External rise of KNO <sub>3</sub> in cm.
1.	6.0	5 mins.	4.8
2.	15.1	15 mins.	7.2
3.	9.3	30 mins.	Tip
4.	15.6	1 hr.	Tip

*Amount of external conduction.*

Table VII shows that the amount of liquid conducted externally is very large, 1.7 c.c. of a 0.5 per cent. solution of sodium chloride being conducted over the external surface of a plant 4.4 cm. long in one day.

TABLE VII.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	3.8	1 day	1.37
2.	4.4	"	1.7
3.	4.1	5 days	3.5
4.	7.5	"	5.54

*Rate of internal conduction.*

Table VIII gives typical results of experiments carried out to determine the rate of internal conduction.

TABLE VIII.

Readings for potassium nitrate in cm.					Readings for lithium sulphate in cm.		
Plant number.	Time.	Length of plant.	Region submerged.	Internal rise of KNO <sub>3</sub> .	Length of plant.	Region submerged.	Internal rise of Li <sub>2</sub> SO <sub>4</sub> .
1.	1 hr.	4.7	0.9	0.4	4.9	0.4	0.8
2.	2 hrs.	5.0	1.1	1.3	5.0	0.3	0.9
3.	3 hrs.	5.1	1.3	1.5	4.9	0.4	1.2
4.	4 hrs.	5.0	1.3	1.6	5.8	0.5	1.4
5.	18 hrs.	5.5	0.6	2.2	5.2	0.3	1.9
6.	24 hrs.	4.3	0.2	1.5	5.0	0.6	1.6

Although internal conduction does take place in this moss it is evident that the rate at which it occurs is slow and therefore this means can only be of secondary importance to the plant.

*Internal anatomy of the stem and leaf of Dicranum scoparium.*

An examination of sections of the stem revealed an epidermis and outer cortex of thin-walled cells which become thick-walled and pitted in the older parts of the plant; a thin-walled, large-celled inner cortex; and a central strand which occupies about one-tenth of the diameter of the stem and which consists of thin-walled, elongated cells with oblique walls (Figs. 5 and 7).

The midrib of the leaf is composed of a central layer of large, thick-walled cells which are continuous laterally with the cells of the leaf-blade and which are surrounded by smaller, thick-walled cells (Fig. 6). The midrib terminates in the hypodermis of the stem.

*Entry of materials and path of internal conduction.*

The entry of materials is, as might be expected, most rapid and most frequent in the region of the thin-walled peripheral tissue at the apex of the stem, where penetration occurs within half an hour of placing the base of the plant in solutions. Lower down on the stem the rate of penetration is so slow that often no indication of the presence of solutions in the inner cortex was obtained for two or three days after the immersion of the base of the plant in the solutions. Any internal conduction is also slow and can be related to the lack of differentiation in the cells of the stem.

III. *Hylocomium triquetrum.*

This moss occurs commonly on the ground of woods and hedges, but the specimens examined in this investigation were collected from the drier regions of dune slacks where they formed large, yellowish mats from six to eight inches high. The stems are erect, stout, and rigid, irregularly but densely branched and covered with large leaves which are widely triangular at the base but taper to an acute apex (Fig. 8).

*Rate of external conduction.*

Typical results obtained in experiments determining the rate of external conduction are given in Tables IX and X.

The following tables show that the rate of external conduction in this moss is exceptionally rapid, in some cases the liquid rising 2 cm. in one minute. This rapid rate of ascent can be correlated with the dense crowding and imbricating arrangement of the leaves.

TABLE IX.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in						
		1 min.	15 mins.	30 mins.	1 hr.	2 hrs.	3 hrs.	6 hrs.
1. (G. violet)	3.97	0.2	3.3	3.46	Tip	—	—	—
2. "	4.24	1.9	2.68	3.5	3.92	4.0	Tip	—
3. (Iron)	3.75	1.2	1.97	2.1	2.15	2.3	2.5	3.0
4. "	5.46	0.1	0.1	1.8	3.0	3.2	3.4	3.6
5. (Acid)	4.5	1.9	3.63	3.9	4.0	Tip	—	—
6. "	5.1	2.0	2.23	2.85	3.7	3.9	4.0	Tip

TABLE X.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	5.3	5 mins.	2.3
2.	6.3	15 mins.	4.8
3.	7.2	30 mins.	5.1
4.	6.6	1 hr.	6.0
5.	5.2	2 hrs.	Tip

*Amount of external conduction.*

The results tabulated in Table XI show that the amount of liquid conducted externally is also very large and is probably sufficient to supply all the water requirements of the plant.

TABLE XI.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	4.8	1 day	1.58
2.	5.1	"	1.1
3.	4.5	5 days	5.15
4.	4.7	"	3.69

*Rate of internal conduction.*

Typical results obtained for the rate of internal conduction are given in Table XII.

TABLE XII.

Plant number.	Time.	Readings for potassium nitrate in cm.			Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	18 hrs.	6.2	0.3	0.7	5.7	0.2	0.4
2.	24 hrs.	5.6	0.2	0.1	4.2	0.2	0.4
3.	3 days	5.8	0.3	0.2	5.6	0.9	0.6
4.	"	5.0	0.3	0.7	4.8	0.7	0.5

It is evident that in this moss again a very rapid rate of external conduction is accompanied by a very slow rate of conduction internally.

*Internal anatomy of the stem and leaf of Hylocomium triquetrum.*

Serial sections of this moss showed little differentiation of tissues in both stem and leaf (Figs. 9 and 10). The epidermis and hypodermis of the stem are composed of small cells which are very thick-walled and sparsely pitted in the older regions of the plant; the cortex consists of larger cells which also become thick-walled; while the central strand is small and consists of from five to twenty small, thin-walled, short cells with numerous transverse walls.

The lamina of the leaf is continuous with the epidermis, while the two small 'midribs' are continuous with the hypodermis of the stem and are composed of cells similar to those of that tissue (Fig. 9).

*Entry of materials and path of internal conduction.*

It is obvious that this moss possesses the capacity to conduct water to the tip of the stem in a very short time, the rate and amount of conduction externally being even greater than that recorded above in view of the naturally densely tufted habit of the plants. The penetration of liquids reaching the tips of the stems is rapid, potassium nitrate having penetrated, in most cases, into all tissues within 1 cm. from the apex of the stem in twenty-four hours. The entry of solutions in regions where the hypodermis is thickened is very slow and the conduction of liquids both upwards and downwards through the internal tissues takes place at only a very slow rate, but is slightly more rapid in the central strand than in the cells of the cortex.

IV. *Ditrichum flexicauli* var. *densum*.

This slender variety of a characteristic species was obtained from the dry regions of partially colonized dunes. The plants grow in bright green, dense, silky tufts from two to five centimetres high, the individual plants being free for the upper third of their length. The stems are very slender and covered with rather long, lanceolate leaves whose pronounced nerves are strongly excurrent (Fig. 11).

*Rate of external conduction.*

The rate of external conduction was investigated for both normal air-dried and very dry, wilted specimens. It was found that in both cases the rate of conduction externally was exceptionally rapid, but was slightly more rapid for air-dried specimens than for very dry forms. Typical results of this investigation are given in Table XIII. Owing to the small size of the

moss and to the rapid rate of conduction accurate readings for potassium nitrate were difficult to obtain and are therefore omitted for this species.

TABLE XIII.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in			
		1 min.	5 mins.	10 mins.	15 mins.
1. (G. violet)	1.9	1.0	1.6	Tip	—
2. "	1.8	0.8	Tip	—	—
3. (Iron)	1.5	1.1	Tip	—	—
4. "	2.0	0.3	0.6	1.2	Tip
5. (Acid)	1.8	1.6	Tip	—	—
6. "	1.6	0.8	1.1	1.4	Tip

*Amount of external conduction.*

Typical results obtained in an investigation of the amount of salts conducted externally are given in Table XIV.

TABLE XIV.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	3.6	1 day	0.12
2.	3.0	"	0.18
3.	2.1	5 days	0.35
4.	2.5	"	0.29

Although the rate of external conduction was rapid the above table shows that the actual amount of liquid conducted is small. It must be borne in mind, however, that this moss has an exceptionally slender stem and the amounts recorded are therefore of considerable importance and will be further augmented in nature as a result of the tufted habit of the plant.

*Rate of internal conduction.*

Table XV gives typical results obtained in an investigation of the rate of internal conduction. It is clear that this slow rate of conduction can be of little importance to the plant.

TABLE XV.

Plant number.	Readings for potassium nitrate in cm.				Readings for lithium sulphate in cm.		
	Time.	Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	24 hrs.	3.0	0.4	0.2	2.0	0.3	0.1
2.	"	3.6	0.3	0.1	3.0	0.4	0.1
3.	3 days	2.3	0.3	0.6	1.7	0.5	0.5
4.	"	2.0	0.7	0.3	1.9	0.3	0.2

*Internal anatomy of the stem and leaf of Ditrichum flexicauli var. densum.*

Serial sections of the stem showed the following tissues: An epidermis and hypodermis of small cells which are thick-walled and pitted throughout the greater part of the stem; a cortex of larger cells and a few narrow, elongated, thin-walled cells forming the central strand (Fig. 12).

Sections of the leaf showed a single-layered lamina of cells similar to and continuous with the epidermis of the stem and a multi-layered midrib composed chiefly of cells similar to those of the hypodermis but with a central row of larger cells comparable to those seen in the midrib of *Dicranum scoparium* (Fig. 12).

*Entry of materials and path of internal conduction.*

An investigation of the mode of entry of materials into the stem showed that penetration occurs most frequently and most rapidly through the unthickened cells at the tip of the plant, the rate of external conduction in this form being sufficiently rapid to ensure a plentiful supply of liquid reaching this tissue. Entry of materials farther down on the stem or at the base is extremely slow, and where penetration of liquid into these tissues does occur the rate and amount of conduction internally is negligible.

*V. Anomodon viticulosus.*

Large yellowish-green tufts of this moss are commonly found growing on old walls and roots of trees. The plants vary from four to eight centimetres in length, the primary stems being stoloniform with secondary erect branches. The leaves are closely placed, widely ovate at the base but tapering gradually to a fine apex and are secund. When dry the leaves become twisted and curled about the single pronounced 'midrib' which extends from the base to near the apex (Fig. 13).

*Rate of external conduction.*

Results obtained in an investigation of the rate of external conduction are given in Table XVI. Readings for potassium nitrate are again omitted for reasons mentioned previously. (See *D. flexicauli*).

TABLE XVI.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in				
		1 min.	5 mins.	10 mins.	15 mins.	1 hr.
1. (G. violet)	3.26	1.9	2.3	Tip	—	—
2. „	3.66	1.6	2.2	3.2	Tip	—
3. (Iron)	2.2	0.9	1.4	1.5	Tip	—
4. „	3.5	0.8	1.0	Tip	—	—
5. (Acid)	1.7	Tip	—	—	—	—
6. „	2.4	0.9	1.5	1.5	1.9	Tip

The rate of external conduction is obviously very rapid, the rate being sufficient in most cases to supply the tips of the plants with water in a few minutes. Where the plants were previously very dry and twisted the initial rate of external conduction was found to be slower, due to the greater length of time necessary to allow an accumulation of water in the leaf axils, but once started the rate was found to be as rapid as that recorded above.

*Amount of external conduction.*

Typical results obtained in an investigation of the amount of liquid conducted externally are given in Table XVII.

TABLE XVII.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	2.6	1 day	0.29
2.	5.1	"	0.41
3.	2.7	5 days	0.87
4.	2.1	"	0.53

The amount of conduction is greater than in the case of *D. flexicauli* var. *densum*, and can be correlated with the larger size of the stem of this moss.

*Rate of internal conduction.*

Table XVIII gives typical results obtained for the rate of internal conduction.

TABLE XVIII.

Plant number.	Readings for potassium nitrate in cm.				Readings for lithium sulphate in cm.		
	Time.	Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	1 hr.	4.2	0.3	0.3	3.7	0.2	0.5
2.	2 hrs.	5.6	0.5	0.75	4.8	0.3	1.3
3.	3 hrs.	3.0	0.2	0.9	3.7	0.2	0.2
4.	4 hrs.	5.9	0.4	0.4	3.2	0.4	0.8
5.	18 hrs.	4.5	0.6	0.8	6.7	0.5	1.3
6.	24 hrs.	3.8	0.2	1.1	3.8	0.3	2.5

The above table shows that the rate of internal conduction in this moss is more rapid than in most of the previous species studied from this third type of habitat, but that the rate is very much slower than that of external conduction.

*Internal anatomy of the stem and leaf of Anomodon viticulosus.*

Serial sections of the stem showed an epidermis and hypodermis of small cells which become thickened rapidly and are very sparsely pitted; a large cortex of thin-walled cells; and a small central strand of thin-walled, narrow, elongated cells (Figs. 14 and 15).

The single-layered lamina of the leaf consists of cells similar to and continuous with the hypodermis of the stem, while the midrib is composed of a very large number of uniform cells which are continuous with the hypodermis of the stem (Fig. 14).

*Entry of materials and path of internal conduction.*

The rapid rate of external conduction in this moss ensures a plentiful supply of water reaching the apex of the plant. Experiment showed that liquid penetrated the internal tissues at the tip at a greater rate than elsewhere on the stem and entry at the base is slow. The rate of internal conduction through the central strand is greater than in the previous moss, but the rate of diffusion both upwards and downwards internally is still very slow.

VI. *Polytrichum commune.*

This moss, which is one of the most common of all species, is found in great abundance on waste ground, sometimes growing in shaded regions, at other times protected by larger grasses around. As has already been pointed out (2), the moss is large, often one hundred centimetres long. The lower 'rhizome' region of the stem is prostrate and closely entwined with its neighbours, whilst the short upper part of the stem is erect and densely covered with large leaves whose long leaf-bases enclose narrow 'pockets' between their adaxial surface and that of the stem.

Experimental work on this moss has been described in detail (2), and the results of these experiments proved that contrary to preconceived ideas the main water-supply of the moss is conducted, not internally through the elaborate central strand, but externally as capillary films of water stretching from the reservoirs in the axils of the leaf-bases, through the narrow channels enclosed between the leaves and the stem, to the tip of the plant. Moreover, the rate of such conduction is rapid, for in some cases the liquid was conducted externally for 12 cm. in one hour. The amount of liquid so conducted appears to be sufficient to supply all the needs of the plant. It was also shown that the penetration of liquids into the stem was most rapid at the apex, but that entry also took place through the leaf-bases and leaf-traces; and that the rapid penetration was associated with definite hydroids in the leaf-trace.

Thus in *P. commune* a greater development is seen both in the



efficiency of the external conducting capacity of the moss as a result of larger and more sheathing bases, and also in the more rapid means of penetration into the internal tissues, whilst the rapid growth of the plant consequent upon the obtaining of adequate water-supply has necessitated the thickening of the walls of the cells of the central strand which apparently serves largely as a supporting tissue.

#### VII. *Mnium hornum*.

The habitat of this species is very varied, but it is commonly found growing on the sloping sides of hedges and on and about the roots of trees, in dense tufts of a dark green colour. The plants are short, rarely reaching a length of more than eight centimetres, but they are firm and erect. The stems are unbranched and closely covered with leaves, those on the lower parts of the stem being small, but increasing in size and becoming more crowded near the apex round the reproductive organs (Fig. 16).

#### *Rate of external conduction.*

The rate of external conduction was measured and typical results are given in Tables XIX and XX.

TABLE XIX.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	3.19	0.83	0.86	0.86	0.91	0.91	0.91
2. "	2.52	0.57	0.67	0.67	0.67	0.67	0.67
3. Iron	4.12	1.17	1.29	1.29	1.3	1.3	1.38
4. "	4.76	0.96	1.0	1.01	1.02	1.04	1.05
5. (Acid)	2.92	0.45	0.5	0.59	0.67	0.67	0.67
6. "	1.52	0.44	0.51	0.53	0.54	0.54	0.62

TABLE XX.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	2.7	1 hr.	1.2
2.	2.5	2 hrs.	1.8
3.	3.5	3 hrs.	2.5
4.	3.8	6 hrs.	2.8
5.	4.8	24 hrs.	4.0

The rate of external conduction in this form is obviously slower than that recorded for most preceding mosses. It must be remembered, however, that these plants normally grow in dense tufts, but experiments carried out with groups of plants showed that even under conditions of normal growth the externally conducted materials did not always reach the tips of the plants.

*Amount of external conduction.*

An investigation of the amount of liquid conducted externally gave results of which Table XXI is typical.

TABLE XXI.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	2.2	1 day	0.26
2.	3.5	"	0.29
3.	1.7	5 days	0.89
4.	2.5	"	0.64

The amount of external conduction is small, and so it must be concluded that although the rate of external conduction of small quantities of liquids is fairly rapid, the amount of such conduction appears in most cases to be insufficient to meet all the plant's demands.

*Rate of internal conduction.*

Table XXII records typical results obtained in an investigation of the rate of internal conduction.

TABLE XXII.

Plant number.	Readings for potassium nitrate in cm.				Readings for lithium sulphate in cm.		
	Time.	Length of plant.	Region submerged.	Internal rise of $\text{KNO}_3$ .	Length of plant.	Region submerged.	Internal rise of $\text{Li}_2\text{SO}_4$ .
1.	1 hr.	2.2	0.3	0.4	3.7	0.5	0.7
2.	2 hrs.	2.8	0.5	0.6	2.8	0.6	1.0
3.	3 hrs.	3.0	1.0	Tip	3.2	0.6	1.3
4.	4 hrs.	2.4	0.3	0.4	5.1	0.4	2.4
5.	18 hrs.	2.5	1.0	Tip	3.4	0.4	2.9
6.	24 hrs.	3.8	0.6	Tip	4.8	0.6	Tip

Although the rate of internal conduction is fairly slow, yet it is sufficiently rapid to supply the tips of plants 4.8 cm. high with water after twenty-four hours, and since this moss grows so prolifically in fairly dry situations it can be concluded that external and internal conduction are together quite sufficient to supply all the needs of the plant and that internal conduction plays a relatively larger part than in the case of other mosses examined.

*Internal anatomy of the stem and leaf of Mnium hornum.*

Figs. 17 and 19 represent a typical section of this moss and the following tissues are seen: An epidermis and hypodermis of thick-walled

pitted cells; a cortex of larger elongated cells with thinner walls; a central strand consisting of a large number of small, long, narrow, thin-walled cells with obliquely-transverse walls. The central strand is larger than in preceding forms and occupies about one-fifth of the diameter of the stem in the upper part, but in the older, lower regions of the stem it is smaller with a corresponding increase in the size of the cortex.

The leaves of this moss (Fig. 18) are large and consist of a single-layered lamina and multicellular, several-layered midrib. The midrib is composed of two kinds of cells; large thick-walled cells, comparable with those of the hypodermis of the stem, and smaller thick-walled hydroids which terminate in the hypodermis or outer layers of the cortex forming leaf-traces. As in *Mnium punctatum* these traces end blindly in the hypodermis and do not transverse the cortex and join the central strand.

#### *Entry of materials and path of internal conduction.*

It was found that where liquids conducted externally reached the tip of the plant the solutions penetrated through all the apical tissues before the liquids conducted internally reached the apex. When the liquids conducted externally did not reach the tip then materials conducted through the central thin-walled cells were the only ones to reach the apex of the plant. Entry through the hypodermis in these cases was relatively slow and penetration was found to take about twenty-four hours. Moreover, it was found that in specimens in which external conduction was rapid, internal conduction was slow, whilst in those cases in which external conduction was slow the supply was augmented by a more rapid internal rise—a fact which can be attributed to the increased osmotic concentration of the cells at the apex, when external conduction is too slow to supply them with water.

#### VIII. *Mnium undulatum*.

This moss is one of the most common forms and is found growing in large, loose patches on hedges and amongst grass on dry soils. The plants are large, often reaching a length of twelve centimetres, with erect stems and large branches which tend to arise at the same level on the stem. The leaves, too, are large, decreasing in size towards the apex, lingulate in shape and attached to the stem by a narrow, slightly decurrent midrib which gives a petiolar appearance (Fig. 20).

#### *Rate of external conduction.*

Typical results obtained in an investigation of the rate of external conduction are given in Tables XXIII and XXIV.

TABLE XXIII.

Plant number.	Length of plant in cm.	External rise of liquid in cm. in					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	24 hrs.
1. (G. violet)	4.5	1.78	1.78	1.9	2.0	2.0	2.1
2. "	8.6	1.0	1.0	1.1	1.14	1.14	1.2
3. (Iron)	8.6	0.9	1.8	2.4	2.6	2.9	3.0
4. "	6.5	2.0	2.5	2.9	3.0	3.0	3.5
5. (Acid)	10.0	0.18	0.18	0.19	0.6	0.7	1.1
6. "	4.5	0.89	1.0	1.2	1.3	1.5	1.5

TABLE XXIV.

Plant number.	Length of plant in cm.	Time.	External rise of $\text{KNO}_3$ in cm.
1.	4.0	1 hr.	1.3
2.	7.5	2 hrs.	1.4
3.	12.0	3 hrs.	3.5
4.	8.0	18 hrs.	3.4
5.	7.5	24 hrs.	4.0

Whilst it is evident that external conduction does take place to some small extent and can be accounted for by the smaller size and denser arrangement of the leaves near the base of the stem, it is quite clear that the rate of this conduction is far too slow to supply the plant with all its necessary water.

*Amount of external conduction.*

Estimates of the amount of water conducted externally are given in Table XXV.

TABLE XXV.

Plant number.	Length of plant in cm.	Time.	Amount of salt solution in c.c. risen over the external surface.
1.	5.4	1 day	0.5
2.	6.0	"	0.17
3.	4.5	5 days	0.95
4.	6.8	"	0.23

The amount of external conduction is extremely small and must be negligible in the drier regions where the larger expanded leaves are exposed to a dry atmosphere.

*Rate of internal conduction.*

The internal conducting capacity of this moss, measured in the usual way is recorded in Table XXVI. It is evident from the table that the rate of internal conduction in this species is far more rapid than for any other form examined.

TABLE XXVI.

Plant number.	Time.	Readings for potassium nitrate in cm.			Readings for lithium sulphate in cm.		
		Length of plant.	Region submerged.	Internal rise of KNO <sub>3</sub> .	Length of plant.	Region submerged.	Internal rise of Li <sub>2</sub> SO <sub>4</sub> .
1.	15 mins.	5.1	0.5	1.1	7.2	0.6	2.1
2.	30 mins.	4.8	0.5	1.9	4.9	0.4	2.6
3.	1 hr.	7.0	0.8	2.5	10.4	0.4	4.2
4.	2 hrs.	10.0	1.0	9.0	6.9	0.6	4.6
5.	3 hrs.	9.0	0.6	1.0	8.6	1.0	6.2
6.	18 hrs.	6.5	1.0	4.5	9.9	0.6	Tip
7.	24 hrs.	9.0	1.3	Tip	9.5	0.5	Tip

*Internal anatomy of the stem and leaf of Mnium undulatum.*

An examination of sections of the stem of this moss showed the following tissues (Figs. 21 and 23): A small-celled, thin-walled epidermis and hypodermis in sections taken near the apex, these tissues farther down on the stem being thick-walled and densely pitted; a large-celled, thin-walled inner cortex; and a large, small-celled, thin-walled central strand which occupies from one-half to two-thirds of the diameter of the stem and is composed of long, narrow cells with few transverse walls as seen in longitudinal section.

The leaf of this moss is large (Fig. 22) and consists of a single-layered lamina and a several-layered midrib. The midrib is large and is composed of about one hundred cells, the central six to twelve of which are small hydroids. The hydroids of the apical leaves are thin-walled and comparable with the cells of the central strand, but in the older leaves, unlike the cells of the central strand, they become thickened in a manner similar to that of the epidermis and hypodermis. The midrib terminates in the hypodermis and outer layers of the inner cortex, the hydroids forming leaf-traces which never become attached to the central strand.

*Entry of materials and path of internal conduction.*

The rate of entry of materials into the stem of this moss, both at the base and higher up the stem, is slow. In some cases the bases of complete plants were dipping into potassium nitrate solution for three and six hours before the solution could be demonstrated in the cortex. Where penetration had occurred, however, conduction through the central strand was rapid. In *Mnium undulatum* a greater development of an internal conducting system is reached than is found in any other moss examined, and the efficiency of this method of conduction appears to be correlated with a diminution of the power of the plant to conduct water externally.

#### DISCUSSION AND CONCLUSION.

From all the results obtained in the investigation recorded in Parts I, II, and III of this work, it is evident that, in general, the moss plant possesses the capacity to conduct water over the external surface and to absorb this water through the unthickened external walls which are generally to be found in the cells congregated at the apex of the plant and in the leaves and branches. At the same time, in all forms examined, certain quantities of water are evidently transported up the central tissue of the stem, though in many cases the rate of such transport is so slow as to render the quantities so raised negligible in comparison with the total water requirements of the plant. In such cases it was found that this very slight internal conduction was correlated with either (*a*) a very marked external conduction which would supply the plant with all the water it needs, or (*b*) a habitat so moist that atmospheric water would condense on the whole surface of the plant in such quantities as to obviate any need for conduction at all.

This is to be expected in view of the fact that the mosses are presumably descendants of submerged thallophytic ancestors which have adopted a more or less subaerial habitat. These ancestral forms would absorb water containing the necessary nutrient salts over their whole surface, and their thalloid descendants, migrating to the land, might naturally be expected to retain this characteristic, which would enable prostrate thalli in moist land habitats to satisfy all their requirements. The finer the branches of such thalli remained, the greater would be their surface in proportion to the area of their transverse section, and the greater would be their power of retaining external films of water as a result of surface tension. As these branches became erect, these films adherent to them would rise to a certain distance above the soil, and if the external walls remained unthickened, such water would be absorbed into the interior. With the occurrence of leaves or other outgrowths on the lower regions of the stem, surface films reaching them would be held by capillarity between them and the stem, and this force of capillarity would be the greater the closer the convergence between outgrowth and stem. The most common of such outgrowths are leaves, and a rapid succession of such leaves, with or without their decurrent leaf-bases which would form pockets and reservoirs in which rain-water would collect, would enable water to rise in the form of a series of capillary films over the whole external surface, until the tip of the plant is reached. This tip being actively concerned with growth would obviously remain unthickened and therefore rapid penetration of water into the apical tissues would occur. This ascent of water to the tip and its penetration into the apex, would negative the effect of the impermeability of the outer walls of the older, and therefore

the lower, parts of the stem consequent upon the thickening which would occur in them as a necessary support and protection as they became erect.

The water conducted over the external surface was not found to be absorbed exclusively by the cells at the apex, for the leaf-laminae were able to supply their needs from these water films, and a certain amount diffused, though in most cases slowly, through the pitted walls of the epidermis and hypodermis in the older parts of the stem.

The above suggestion as to the general scheme of external conduction is supported by the work of Davy (5), who found that submergence in water or growth in a saturated atmosphere tended to increase the growth of moss plants and increase the length of the internodes of the stem. The converse might therefore be expected to be true, i.e. that where water-supplies were scarcer a closer arrangement of the leaves was advantageous in retaining surface films of water.

This was borne out by the examination of the form and leaf arrangement of mosses growing in habitats differing in their water-content and by results obtained from investigations of the external conducting power of these species. Of the species studied *Brachythecium rutabulum* and *Philotis fontana* grow in very humid atmospheres and thus adopt the nearest approach to an aquatic habitat. As such, these forms exhibit a very feeble power of conducting solutions externally, for it is evident that sufficient moisture is present in the atmosphere to condense on the surface of the plants and to supply all their needs.

It is obvious, however, that such moist habitats as these are very limited in extent, and plants requiring such a moist environment are correspondingly restricted in range. Any plant which developed means of conducting water upwards from the soil surface would therefore stand a greater chance of success in a drier habitat, since it would no longer be entirely dependent on water condensed from a humid atmosphere, and such a plant would therefore be enabled to spread more freely.

It is suggested that from, presumably, the more primitive form of Musci which absorb over their whole surface but do not conduct there have been derived the apparently more specialized forms suitable to a drier habitat, and therefore having the power of conducting water in an upward direction. Mosses, such as *Hypnum Schreberi*, *Thuidium tamariscinum*, &c., exhibit an increased power of conducting water as compared with *Brachythecium rutabulum* and this power is apparently due—

(1) To the presence of numerous, branched hair-like processes or paraphyllia which arise from the epidermis of the stem and enclose small spaces between their surface and that of the stem through which capillary films of water may rise.

(2) A denser arrangement of leaves on the stem and the closer proxi-

mity of these leaves resulting in the formation of an increased number of narrow capillary channels per unit length.

In these mosses the leaves are always small and densely packed, the small reservoirs in the axils of the leaves renewing supplies for the rising capillary films. Moreover, the stems of these species are often inclined to the vertical instead of being absolutely erect, so that the rise of water externally is less restricted by the pull due to gravity.

Two further modifications are seen in forms which inhabit still drier environments.

(1) The leaf-bases tend to become more strongly decurrent, as in *Dicranum scoparium* and *Polytrichum commune*, for in this way the channels enclosed between the leaf-bases and the stem are long and narrow, and in *Polytrichum* definite pockets are found in which water collects and is stored.

(2) The second modification is the tufted habit which these forms assume, for by so doing the intertwining of the separate plants ensures the retention of rain-water in the unexposed regions of the plants, and also the added support which the plants receive enables them to grow upwards into better assimilating regions.

The success of the external conducting system as compared with the elaborated internal conducting system of the vascular plants is doubtful, for it imposes important restrictions on the plant such as (a) a restricted growth, for the plants could never increase in height beyond the point to which capillary films could rise, and (b) a restricted supply of nutrient materials, for the greater amount of water for external conduction is obtained directly from the atmosphere and is therefore poorer in dissolved materials than is soil water.

The present work has shown, however, that external conduction is not the only means of conduction in the group, and especially is this so for the genus *Mnium*. It is interesting to note that of the three species of this genus studied the leaves are large and set relatively far apart on the stem. In *M. punctatum*, however, the leaves do slightly encircle the stem, and this fact, together with its tufted habit and profusion of tomenta, enables the plant to conduct some water externally. In *M. hornum* the area of insertion of the individual leaves is smaller, but they are more crowded on the stem, and this fact in addition to the more tufted habit of the plant ensures that here again external conduction takes place. In *M. undulatum*, however, the leaves are large, far apart, and attached by definite 'petioles' and, moreover, the individual plants are quite separate. Here, therefore, external conduction is restricted to a very small region at the base of the plant. Though external conduction is slow and restricted, this genus is provided with a means of internal conduction more efficient than that in any other moss examined.



The power of internal conduction of the mosses examined must therefore be reviewed. In *B. rutabulum* and *H. cupressiforme* var. *filiforme* the power of internal conduction is very limited, and this fact can be correlated with a feeble development of the central strand, which in these mosses consists of a few thin-walled, elongated cells. In *B. purum* and *H. Schreberi* there are a larger number of cells forming a central strand, and here there is a corresponding increase in internal conducting efficiency. The central strand of *M. hornum* is large and consists of extremely elongated cells, but experiment showed that where external conduction was rapid in this moss, the central strand showed a very slow rate of conduction. *M. undulatum*, however, has a very limited external conducting capacity, and this is correlated with a greater power of internal conduction. The number of cells in the central strand is greater than in the other species, and also these cells are longer, so that in longitudinal section they appear as long, narrow, thin-walled vessels. Haberlandt demonstrated an exceedingly rapid rate of internal conduction in this tissue, and the writer has recorded a rise of eight and nine centimetres in one hour, although the rate of entry of solution into these cells is slow. Thus in the gametophyte of *M. undulatum* there is a development of an internal conducting capacity which is comparable with that of the slower conducting wood vessels of Angiosperm.

The leaves of the species studied also show a variation in the degree of differentiation of their tissues. The leaves of *H. cupressiforme* var. *filiforme* and *B. rutabulum* are single-layered throughout, or, if the midrib is present, it is short, double, feebly developed, and restricted to the base of the leaf. In other forms such as *P. fontana*, &c., the midrib is always present and consists of a number of cells usually all the same size which terminate in the hypodermis of the stem. In the genus *Mnium* there is a greater differentiation in the midrib, which here consists of relatively large cells surrounding smaller hydroids. Although these hydroids are present in the three species of *Mnium* examined, in no case were they seen to pass through the cortex and unite with the central strand of the stem. In all cases they terminate in the outer cortex or outer layers of the inner cortex where they appear as definite leaf-traces. A more complex arrangement is seen in *P. commune* where the hydroids of the leaves pass through the outer cortex to the innermost layer of the inner cortex where they fuse to form the hydrom mantle. Tansley and Chick have suggested that the development of the conducting cells of the leaves in the Musci has taken place independently of the development of the conducting tissue of the stem. This suggestion is borne out by the examination of the species studied, and it seems likely that the separate development of the two conducting tissues is definitely related to the low growth and moist environment of the group and also to the external path of water conduction which these plants have adopted. It seems possible that in species of *Mnium*,

with their more marked power of internal conduction, the quantity of water thus obtained, diffusing out to the outer cortex, became sufficient to supply the leaves necessitating the development of the leaf hydroids which terminate in the outer cortex, so that in *M. undulatum* external conduction has been practically eliminated.

It is evident from all the above work, that the limited growth and habitat of the gametophyte generation of the mosses are largely conditioned by the difficulty which these plants encounter in transporting water internally, owing to the lack of an effective conducting strand; but this deficiency is counteracted in many forms by a marked ability to transport water over the external surface by the aid of the closely arranged leaves which retain capillary films, this water being absorbed largely through the thin-walled cells at the apices of the plants and on the leaves and branches, and transported downwards rather than in an upward direction.

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#### SUMMARY.

1. Eight species of Musci were selected from dry habitats and their method of obtaining water investigated.
2. The external morphology and habit of the plants were studied in connexion with their capacity to conduct water externally and there was found to be a correlation between the two.
3. The internal structure of the stems and leaves of the species under investigation was examined, and the paths of internal conduction and of entry into the stem tissues of externally conducted liquids were determined.
4. With the exception of the genus *Mnium*, in all forms examined the amount of water conducted over the external surface exceeded that conducted internally.
5. The water conducted externally ascended in the form of capillary films between the leaves and the stem, and was absorbed by the unthickened cells at the apex of the stem and in the leaves and branches, and diffused through the internal tissues in a lateral and downward, rather than in an upward, direction.
6. It was found that water ascending internally travelled through the narrow, elongated, thin-walled cells of the central strand.

7. The genus *Mnium* shows the greatest differentiation of internal tissue in the stem, and also the presence in the leaves of definite conducting hydroids, which are absent from the leaves of all other forms examined. *Polytrichum*, whose marked power of conducting water externally has been fully reported in a previous communication, is an exception to this statement.

8. It was found that, in general, the power of the plant to conduct water both externally and internally diminished as the moisture content of the habitat increased, presumably owing to the fact that the moister the habitat the more water to supply its needs would be deposited from the humid atmosphere over the whole surface of the plant.

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## NOTES.

### ON THE PRESENCE OF CITRININ IN CROTALARIA CRISPATA.

F.v.M.—This plant is fairly widely spread in North Australia, extending from N.W. Australia, sandy rises near the Fitzroy river, and inland from Broome and Derby to the Victoria river, islands of the Gulf of Carpentaria and Northern Queensland. It is a low, much branched herb, under a foot high, covered with hairs, the leaves of a yellowish colour, oblong, unifoliate, rarely trifoliate. It differs from most *Crotalaria*s such as *C. Novae-Hollandiae* and *C. retusa* in hardly darkening when dried, and in not turning black when crushed and exposed to air.

The dried material when crushed yields a yellowish powder, and gives an acid extract with water containing water-soluble saponin. It also contains a rather large proportion of acid saponin soluble in dilute alkali, and a yellow pigment soluble in dilute alkali and in alcohol. The pigment turns brownish red with alkali, pale yellow with acid, and cotton threads stained with the dye can be used instead of litmus paper as an acid-alkali indicator.

When investigating the cause of 'Walk-about' in horses, or Kimberley horse disease, which was found to be due to the prolonged ingestion of plants rich in active saponin, particularly *Atalaya hemiglauca* or whitewood<sup>1</sup> in North Australia, several species of *Crotalaria* were investigated, and bulk specimens collected, dried, and brought to Melbourne for further investigation.

The yellow pigment of *C. crispata* occurs diffusely distributed in the parenchyma of the leaf, but also on the surface as irregular or rounded small particles, especially on the surface of the numerous hairs. In its acid form, which is insoluble in water, the pigment does not appear to undergo photo-chemical oxidation readily, and hence it is possible it may serve to protect the chlorophyllous cells of the leaf from exposure to strong sunlight.

Samples of the yellow pigment obtained in pure form by the methods described beneath were found to be identical with the citrinin obtained by Hetherington and Raistrick<sup>2</sup> as a metabolic product of *Penicillium citrinum* Thom from glucose. The pigment from *Crotalaria crispata* was an acid, insoluble in cold water, but dissolving in dilute sodium carbonate with an effervescence of carbon dioxide, and producing a reddish-brown or orange-yellow solution according to dilution. It forms a pale-yellow solution in watery sodium acetate, and is precipitated from its alkaline solution by acids, such as dilute hydrochloric or acetic acid. It is soluble in chloroform, acetone, hot alcohol, and warm ether, less soluble in cold alcohol, and hardly in cold ether.

<sup>1</sup> Murnane and Ewart, Bulletin 36. C.S.I.R., Melb., 1928. Ewart, Bulletin 50, C.S.I.R., Melb., 1931.

<sup>2</sup> Phil. Trans. B. vol. 220, p. 269, 1931.

It crystallizes from alcohol in golden-yellow prismatic needles, and the alcoholic solution is laevorotatory. It melts at  $168^{\circ}\text{C}$ . with decomposition. A neutral solution gives a dark buff colour with ferric chloride, and with excess a turbid brown solution.

With a solution of iodine in caustic soda, an alkaline solution of the pigment gives an abundance of iodoform with or without gentle warming.

An alcoholic solution of the yellow pigment, to which water was added, was slowly decolorized by zinc dust out of contact with the air, and the decolorization was hastened when the solution was acidified. On shaking with air the yellow colour rapidly returned.

The identity of the yellow pigment of *Crotalaria crispata* with the citrinin formed by *Penicillium citrinum* as a metabolic product from glucose seems, therefore, to be definitely established. The optimum temperature for the formation of citrinin by *Penicillium* appears to lie between  $28^{\circ}\text{C}$ . and  $32^{\circ}\text{C}$ . *Crotalaria crispata* grows in the tropical belt of Australia with an average winter temperature of  $22^{\circ}\text{C}$ . and summer of  $32^{\circ}\text{C}$ ., i.e. at optimal temperature for citrinin production in *Penicillium*. I have examined a number of likely plants from the temperate climate of Victoria for the presence of this pigment, but with consistently negative results, so that it is possible that one condition for the production of citrinin in the sugar metabolism of a flowering plant may be exposure to continuous high temperatures.

*Extraction of citrinin from C. crispata and its estimation.*

The chief difficulties are due to the presence of chlorophyll dissolved with the citrinin if alcohol or ether is used as a solvent, and of acid saponin and chlorophyll extractives which are present if alkali is used and the citrinin (and saponin) precipitated by acid.

In a preliminary test 5 grm. of dry leaf powder were extracted with dilute alkali, and the filtrate acidified. The precipitate was brownish-yellow and contained acid saponin. The yield was 2 per cent.

If a minimum of sodium carbonate is used this is decomposed, forming the soluble sodium salt of citrinin, and insufficient is left to dissolve the acid saponin appreciably. Thus 20 grm. of dry powdered leaf were digested in 2 grm. of sodium carbonate dissolved in 50 c.c. of water, the liquid expressed by pressure, and the residue digested in a further 50 c.c. of water and the liquid again expressed. The total filtered liquid was acidified. The precipitate was pure yellow, and represented a yield of 1.4 per cent. of nearly pure citrinin. It can be further purified by extraction with warm ether, but not without loss.

10 grm. of dry leaf powder were completely extracted with warm ether in a Soxhlet. The extract was evaporated to dryness and dissolved in a minimum of hot alcohol. On cooling, a yield of 0.52 per cent. of pure citrinin separated. The cold alcohol, however, contained much citrinin which could not be separated from the chlorophyll extractives without much loss. The total yield was 1.88 per cent., including decomposition products of chlorophyll.

The best mode of quantitative extraction was found to be as follows:—20 grm. of dry leaf powder were extracted completely with ether in a Soxhlet, and the extract evaporated to dryness. The dry residue was broken up and digested three times with 25 c.c. of 1 per cent. sodium carbonate, warming during the third extraction.

This extract will not filter clear, it is precipitated with acid and the dried precipitate redigested with sodium carbonate. It now filters clear, is reprecipitated with acid, the precipitate collected, dried, and weighed as pure citrinin, brown in mass, yellow when powdered. The yield varied from 1.06 to 1.21 per cent.

The residue from the ether extraction contained water soluble brown extractives and 0.9 per cent. of acid saponin soluble in dilute alkali and precipitated by acid, the water soluble brown extractives tending to cling to the saponin during the early stages of purification.

*Summary.*—The pigment citrinin discovered in Scotland in 1931 as a metabolic product of *Penicillium citrinum* is also produced in a flowering plant, *Crotalaria crispata*, growing in tropical North Australia. It is produced in the leaves and excreted on the hairs, possibly having a biological function as a protection against insolation. The percentage in the dry leaf varies from 1 to 1.2 per cent. It does not appear to occur in any flowering plants growing in the colder climate of Victoria.

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**ON CERTAIN FOSSIL PLANTS FROM EAST AFRICA.**—The object of this note is to place on record certain fossil dicotyledonous woods from East Africa, descriptions of which were not included with those of Dr. Felix Oswald's specimens given in a recent paper.<sup>1</sup> The present collection originally comprised a large number of fragments, very imperfectly preserved in a medium, the condition of which suggests considerable re-crystallization. The majority of these specimens were discarded as unsuitable for cutting; sections of eleven specimens have, however, been examined by the writer, and in nine of them the general character of the structure is indicated, though preservation is not in any instance good, and frequently much displacement of the tissues seems to have taken place.

The specimens are the property of the British Museum (Natural History), to which they were presented by Mr. E. J. Wayland.<sup>2</sup> They were collected by Mr. Wayland himself, and by Dr. H. L. Gordon, Mr. L. Gordon, and Miss Gordon, at Koru, in Kenya Colony (S. 0° 11' by E. 35° 17'), mainly on Dr. Gordon's Medical Farm. The typical Koru strata are fossiliferous Oligocene or Lower Miocene deposits; associated with these are volcanic tuffs, from which the fossils under consideration were obtained.<sup>3</sup> It is impossible to say what is the exact age of these tuffs, or how they came to contain the plant-fragments; these may, of course, have been derived from older strata, and their horizon must be left an open question.

One of the nine specimens (v. 23315) shows the structure of a typical dicotyledonous tree-wood. It is only a fragment, giving no indication of the size of the

<sup>1</sup> Bancroft, H.: Some Fossil Dicotyledonous Woods from the Miocene (?) Beds of East Africa. *Ann. Bot.*, xlv. 745, 1932.

<sup>2</sup> The writer is indebted to Dr. W. D. Lang, the Keeper of the Geological Department, and to Mr. W. N. Edwards, for the opportunity to describe these specimens.

<sup>3</sup> Wayland, E. J.: Annual Report of the Geological Survey of Uganda for 1927, 7, 1928.

original stem; and seasonal zoning is not evident. The vessels are small, and not very numerous: they are arranged singly, in pairs, and in radial groups of as many as five (Fig. 1); and there is a tendency for the tangential diameter of the vessels to be slightly greater than the radial, even where they are singly placed—this may, or

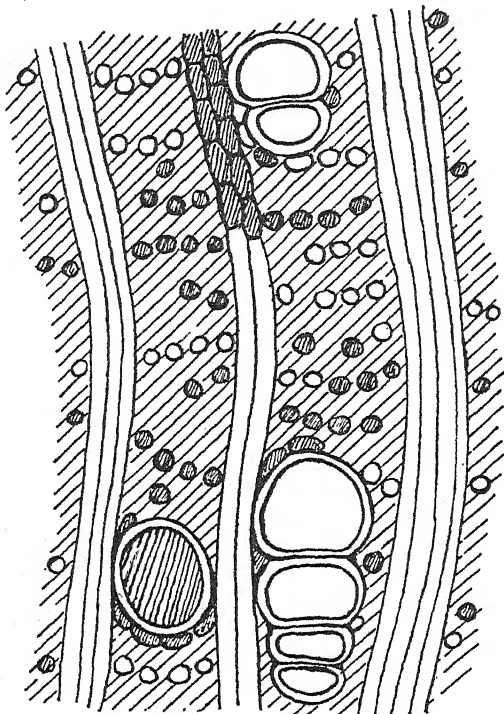


FIG. 1. *Droyoxylon*, sp.: (transverse section). Diagram illustrating the general type of structure of v. 23315. Note the arrangement of the vessels, and the occurrence of irregular uniseriate lines of parenchyma, and also of paratracheal parenchyma. The outlines of the ray cells are only occasionally distinguishable; and those of fibres are entirely obscured; owing to faulty preservation, the exact amount and distribution of parenchyma are difficult to represent. Ray cells, parenchyma, and vessels frequently contain a secretory substance, which is indicated by shading. ( $\times 85$ .)

may not, be natural, since the material is somewhat distorted. From the longitudinal sections it is clear that the vessels are completely perforate, with only slightly inclined ends; they are fairly thick-walled, and in one or two cases the walls are seen to possess small, closely set bordered pits. The vessel-elements, it may be noted, tend to form longitudinal series of considerable extent, and they very generally contain a golden-brown secretory substance. The rays are very numerous and closely set; it is impossible to count the number of radial rows of elements between each two. They are narrow (Fig. 1), and from the very imperfect tangential section they appear to be fairly uniform in width, being typically 3- or 4-seriate. They are not, on the whole, very deep, though there is a good deal of variation in this respect, so that storeying, if present—and there are indications of it—is generally obscured. From both tangential and radial sections the rays appear to be hetero-

geneous. A very characteristic feature of the wood is the distribution of the parenchyma, which occurs in uniseriate, closely set, tangential lines of varying regularity (Fig. 1); a certain amount of this tissue also accompanies the vessels. The longitudinal sections are too imperfect to indicate whether the parenchyma is storeyed or not, or to show how many cells occur in a strand or 'cambiform row'. Both parenchyma and ray cells, like the vessels, frequently contain a secretory substance; but in neither case could any crystal-bearing cells be detected, though the very crystalline condition of the petrifying medium would naturally obscure such structures, even if they were present. Between the tangential lines of parenchyma are thick-walled fibres, the details of which are obliterated in all sections.

The state of preservation of this wood does not allow of detailed comparison with other types, recent or fossil. On account of its general structure it may be included in the form-genus *Dryoxylon*, but it is not advisable to apply a specific reference to wood so imperfectly preserved.<sup>1</sup> In transverse section, v. 23315 shows characters very similar to those of *Drypetes* spp. (Euphorbiaceae) and *Dryoxylon drypeteoides* (see footnote 1), although the tangential lines of parenchyma are perhaps less regular and closely set. The small amount of evidence from the tangential section, however, entirely precludes *Drypetes* from comparison with v. 23315, since the rays of the two types are of totally different character and composition. The nearest approach to the structure of v. 23315 was found in species of *Heritiera* (Sterculiaceae), particularly *H. papilio*. *Heritiera* shows vessels very similar in character, arrangement, grouping, and length to those of v. 23315; close uniseriate tangential lines of parenchyma, separated by thick-walled fibres; rays of a similar width, variation in depth, and degree of heterogeneity to those of v. 23315; and secretory contents in vessels, rays, and parenchyma. So far as these characters are concerned, v. 23315 may be said to be of the *Heritiera* type of structure. *Heritiera*, however, like its very similarly constructed relative *Tarrietia*, is definitely characterized by the possession of crystal-bearing parenchyma,<sup>2</sup> although crystals do not appear to be abundant in *H. papilio*; while in the case of v. 23315 its state of preservation and its extremely crystalline medium, as noted above, render it impossible to make any statement concerning the presence or absence of crystal-bearing parenchyma. Moreover, the parenchyma strands of *Heritiera* are from 2- to 4-celled, and no evidence concerning this point can be obtained from v. 23315.

The similarity of v. 23315 to recent timbers must, therefore, be left undecided; and the specimen is referred simply to *Dryoxylon* sp., and diagnosed as follows:

A dicotyledonous wood from Koru, in Kenya Colony. Vessels of small diameter, singly placed, or in radial groups of 2 or more; short, completely

<sup>1</sup> The writer of this note is now of the opinion that, except in very rare instances, specific names should be given only to fossil woods of which at least the tangential sections, as well as the transverse, yield definite diagnostic data. This applies particularly to those cases where specific names indicating comparison with some recent type are employed. The writer would now, for example, hesitate considerably before naming the British Museum specimen v. 21363 '*Dryoxylon drypeteoides*', on the grounds of similarity of its transverse section alone to those of *Drypetes*, spp. (See Ann. Bot., xlv, 761-4; Text-fig. 4; Pl. XXIX, Fig. 8.)

<sup>2</sup> Chataway, M.: The Wood of the Sterculiaceae: I. Specialization of the Vertical Wood Parenchyma within the Sub-family Sterculiaceae. New Phyt., xxxi, 119, 1932. See p. 130.



perforate, and with fairly thick walls having small, closely set bordered pits. *Rays* numerous; narrow, very typically 3 or 4 cells wide; variable in depth; heterogeneous. *Parenchyma* characteristically arranged in uniseriate, closely set tangential lines. *Fibres* thick-walled. Vessels, ray cells, and parenchyma very generally containing a *secretory substance*. An imperfectly preserved wood, reminiscent in certain respects of the *Heritiera* (Sterculiaceae) type of structure.

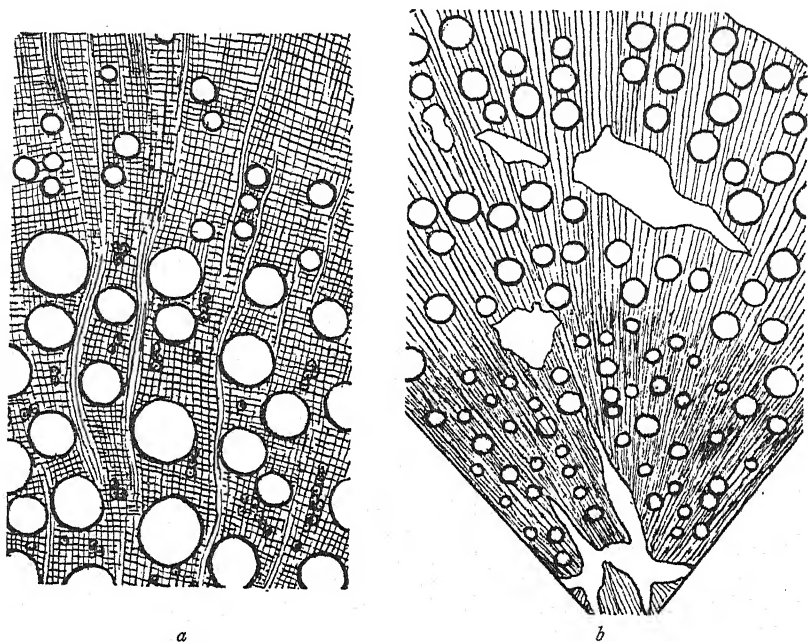


FIG. 2. *Helictoxylon*, sp. (transverse sections). *a*. Diagram of the structure of v. 23313, showing an area of larger and more numerous vessels followed by one with fewer and smaller vessels. The indistinct rays and small-celled ground-tissue are indicated purely diagrammatically; a few isolated and grouped fibres are shown in the ground-tissue, but owing to imperfect preservation, it is impossible to say whether or not more fibres are actually present. ( $\times 27$ ). *b*. Diagram of the structure of v. 23314, showing the central portion of a vascular cord, with what appears to be central and periaxial secondary wood. The denser nature of the former, with its somewhat smaller vessels is indicated. ( $\times 11$ ).

The remaining eight Koru specimens are even less favourably preserved than *Dryoxylon* sp., frequently showing crushing and distortion of the tissues. They are all of the same type, and most probably represent the same species. They show distinctly one main outstanding feature: namely, the possession of numerous large vessels, rounded in transverse section, and typically singly placed (Fig. 2, *a*), though an occasional grouping occurs. Vessels of this type and arrangement are characteristic of lianes in general, and there is thus no difficulty in referring these specimens, in spite of their unfavourable condition of preservation, to the form-genus *Helictoxylon*.<sup>1</sup> Transverse sections of two were finally selected for description—v. 23313 and v. 23314; in no case were the longitudinal sections of any value.

<sup>1</sup> Felix, J.: Studien über fossile Hölzer. Inang. Diss., Leipzig, 1882, 41.

The specimens are so fragmentary that it is not possible to say whether the original stems possessed more than one 'vascular cord'; while only in one instance (v. 23314) is there any evidence that the fragment comprises the central part of the cord, and even here the tissues are so ill-preserved and distorted that no idea of their nature is given, the primary wood not being distinguishable; the central and the periaxial secondary wood are, however, indicated (Fig. 2, *b*), the vessels of the former being somewhat smaller than those of the latter, and the whole central region appearing more dense in texture than the outer part of the wood, apparently possessing a greater proportion of fibres. No grooving or fluting of the wood is indicated, even in the larger specimen, v. 23313; but it cannot be determined whether these fragments represent the central portions of vascular cords which *remain normally and equally thickened throughout*, and are therefore always cylindrical in form, as, for example, in *Thinovia mucronata* (Sapindaceae);<sup>1</sup> or whether they represent the normally thickened central portions of vascular cords which are *ultimately unequally thickened*, and are therefore ultimately ribbed or fluted in outline, as in *Heteropteris intermedia* (Malpighiaceae),<sup>1</sup> or in certain Mimosoideae,<sup>2</sup> and also in forms more complicated in their anatomy than these, such as some of the Bignoniaceous lianes.<sup>3</sup> It is therefore impossible to give any idea, according to Schenck's classification, of the type of liane represented by these specimens.

In all cases the rays are considerably deflected by the vessels, they are exclusively narrow, so far as can be determined from the fragmentary nature of the material, being apparently from 1- to 3- or 4-seriate, as in the case of the Sapindaceous and Malpighiaceous lianes;<sup>4</sup> they are, however, somewhat difficult to distinguish from the small-celled ground-mass of the wood, the exact composition of which, owing to defective preservation, cannot be determined: the impression is given of a relatively high proportion of regularly arranged parenchyma, with fibres isolated or in small irregular groups. Specimen v. 23313 is a fragment of wood in which the vessels are large and numerous in what is apparently an inner layer, towards the centre of the vascular cord, and smaller and much less numerous in an outer layer, where the small-celled ground tissue is more evident, and where it appears to be mainly parenchymatous. It is possible that these two layers represent the concentric zones of larger and smaller vessels, reminiscent of annual rings, such as Schenck notes in *T. mucronata*.

Owing to lack of data, it is impossible to compare these specimens with any one living form of liane; and it is equally impossible, for the same reason, to compare them with the various fossil lianes included in the form-genus *Helictoxylon*, most of which are themselves of very uncertain affinity.<sup>5</sup> Under the circumstances, it is not

<sup>1</sup> Schenck, H.: Beiträge zur Biologie und Anatomie der Lianen. II. Anatomie. Jena, 1893. 84, Pl. III, Fig. 29 c.

<sup>1</sup> Schenck., Pl. VI, Fig. 58.

<sup>2</sup> Ibid., Pl. X, Fig. 135. Solereder notes that this figure probably represents a species of *Acacia* (see 'Systematic Anatomy of the Dicotyledons', 1908, 299).

<sup>3</sup> Ibid., Pl. XI.

<sup>4</sup> Solereder, l.c., 232 and 164.

<sup>5</sup> Edwards, W. N.: Fossilium Catalogus. II. Plantae. Pars 17: Dicotyledones (Ligna), 1931. See pp. 44 and 45. References to other lianes more definitely named by reason of their structure

advisable to do more than refer specimens v. 23313 and v. 23314 to *Helictoxylon* sp., the diagnosis of which is as follows:

The fragmentary wood of a fossil liane, from Koru in Kenya Colony. Central and periaxial secondary wood distinguishable in one specimen. *Vessels* of the periaxial secondary wood typically large, numerous and singly placed, but occurring apparently in zones of larger and more numerous, and smaller and less numerous, examples. *Rays* apparently exclusively narrow, from 1- to 3- or 4-seriate; not easily distinguishable from the ground-mass of the wood. *Ground-mass* small-celled, giving the impression of being largely parenchymatous. More fibres appear to be present in the central than in the periaxial secondary wood.

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March, 1933.

**THE NATURE OF SALTATION IN CERTAIN SPECIES OF HELMINTHOSPORIUM AND FUSARIUM.**—During a recent investigation on hyphal anastomosis, some observations were made which throw light on the nature of saltation in certain fungi. A statement of the results of these experiments may be of interest to botanists as well as mycologists. The full description is being published as a Bulletin of the University of Minnesota.<sup>1</sup>

The organisms used were three species of *Helminthosporium*: namely *H. pedicellatum*, Henry; *H. monoceros*, Drec; *H. sp.* (*Brachysporium* type); and two species of *Fusarium*: namely *F. fructigenum*, Fries; and *F. vasinfectum*, Atk. var. *lutulatum*.

The results may be divided into two sections, those which prove that heterocaryosis is not responsible for saltation in these particular fungal strains, and those which indicate that the difference between a saltant and its parent strain is not cytoplasmic in nature.

In the first section, cytological preparations showed that, in all the *Helminthosporia* used, the mycelium and spores were multinucleate, but that there occurred a uninucleate stage at the inception of the sporophore. In the *Fusarium* strains all the cells were found to be uninucleate, unless they happened to be cells formed by the fusion of two or more cells, or cells whose nuclei had divided, but in which transverse septa had not yet been formed. The latter were only found in the younger hyphae, and on spore formation.

A series of successive subculture generations were then made from single spores, single-spore segments, and single cells.<sup>2</sup> At the end of this series of subcultures, and when it was considered certain that the cultures had been derived a number of times from single nuclei, the capacity of the strains to saltate on Richards's medium was tested. It was found that saltation occurred freely.

From these observations it followed that, since saltation occurred in strains

may be found on p. 45 (*Hippocrateoxylon*), p. 71 (*Ruyschioxylon*), pp. 18 and 82 (*Ampeloxylon* = *Vitoxylon*), and p. 79 (*Ternstroemiacinium* = *Ternstroemioxylon*); the 'genera' *Anomaloxylon* (p. 19) and *Lillia* (p. 53) also include lianes.

<sup>1</sup> Bulletin 88, University of Minnesota Agricultural Experiment Station, November 1932.

<sup>2</sup> The method of isolation of single-spore segments is described in *Phytopathology*, xxiii. 357-67, 1933.

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